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EXPERIMENTAL AND CLINICAL OBSERVATIONS REGARDING ANGINA PECTORIS AND SOME RELATED SYMPTOMS*

D. E. JACKSON, PH.D., M.D., AND HELEN L. JACKSON, M.D.,
CINCINNATI, OHIO

IN OUR opinion angina pectoris is due to acute, spasmodic, incoordinated contractions of the esophagus and stomach. By this reaction air or other stomach contents are entrapped either in the esophagus or in one portion of the stomach. That such action occurs in the human subject is well demonstrated by the fact that more than forty cases have now been described in the literature in which at autopsy complete rupture of the esophagus has been found. The symptoms in these cases generally resemble those of coronary thrombosis as now usually recognized, and confusion and mistakes in the diagnosis are easily understood. Perhaps the most striking case of rupture of the esophagus was that described (the first on record) by Hermann Boerhaave[1] in the case of Baron de Wassenaer in 1724. In this case the esophagus (so nearly as we can determine) was torn completely in two a little ways above the diaphragm. This indicated an endwise pull (by vomiting in that case) on the esophagus, but the usual rupture is a longitudinal slit, generally on the left side about one inch above the cardiac sphincter. While more than forty of these cases are proved to have undergone *complete* rupture, we have found but little emphasis placed in the literature on what we believe to be a vastly greater number of cases in which partial rupture, or temporary straining, occurred. This is the type of case (the stomach may be included) which we believe represents what is now known as angina pectoris. If there be no rupture either into the lumen of the esophagus or into the pleural cavity, then no visible hemorrhage may be detected even at autopsy. But it was well

*From the Department of Pharmacology of the University of Cincinnati College of Medicine.

known even to Heberden that these patients occasionally vomit and that sometimes the vomitus contains blood. The presence of ulcers or other lesions in the esophagus, gallbladder or stomach does not change the viewpoint, but, only makes more probable the occurrence of incoordination and spasticity through the local nervous mechanism. When pain radiates down one or both arms, then we believe that it originates in that portion of the esophagus and adjacent (strained or injured) mediastinum which lies between the horizontal midline of the heart and the upper opening of the thorax. The evidence for this will appear below. But if the pain remains in the neighborhood

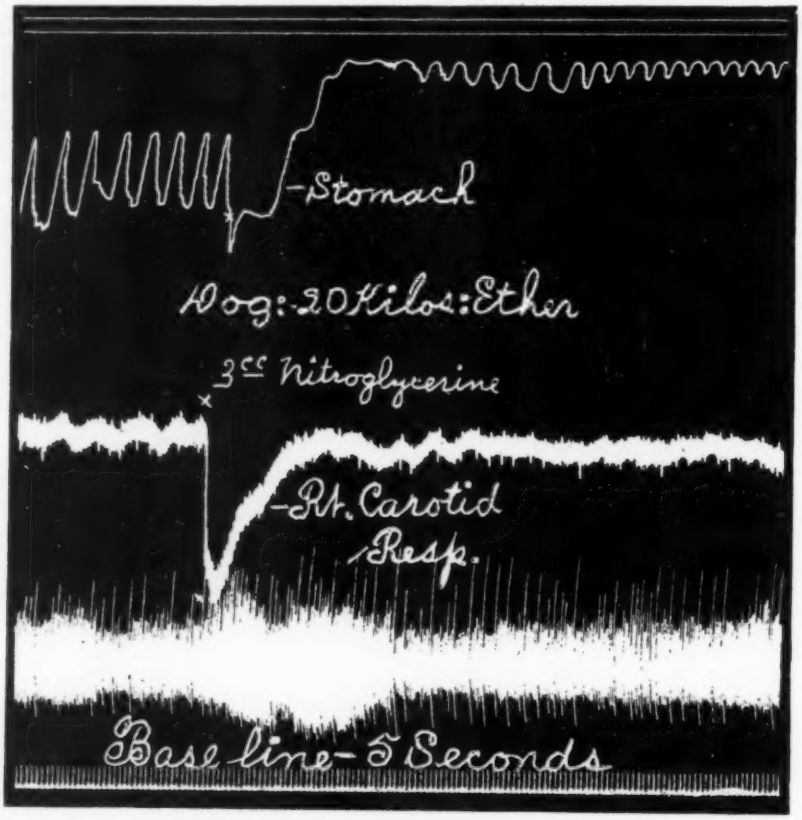

Fig. 1.—Upper tracing, stomach; middle tracing, blood pressure; lower tracing, respiration. At "X" 3 c.c. of nitroglycerine (1/5 per cent) were injected by vein. Stomach contractions immediately ceased and the walls relaxed. This permitted the longitudinal fibers, to which the instrument was attached in front of the greater curvature, to shorten passively. This caused the writing lever to rise, but the stomach as a whole was completely relaxed.

of the ensiform or a little way above and does not radiate laterally (to arms, etc.), although it may extend up into the neck, jaws, and those parts of the face and neck which are innervated by the tenth nerve and the fifth nerve (which has communications with the tenth), then we believe that the origin of this type of pain (probably chiefly vagus) lies mainly in the stomach, or in the stomach and the lower end of the esophagus.

Perhaps not a single extensive article on angina pectoris from Heberden on down has failed to note that the relief of pain in this type of case is often

associated with the eructation of a large volume of gas. Our own observations, both experimental and clinical, lead us to suspect that the relief of the pain really begins a little while before the gas escapes. This is due, in our opinion, to the beginning relaxation of the stomach wall and of the cardiac sphincter and perhaps of contracted segments of the esophagus (in which the longitudinal muscle layer is involved). This permits the gas to escape. That the relaxing action of nitrites on smooth muscle may greatly accelerate this process will be obvious at once to all pharmacologists (Fig. 1). No gas or other stomach contents may escape through the mouth, however, for relaxation of the stomach may permit their painless retention. (This, we believe, has actually been seen fluoroscopically in man by Payne and Poulton.)

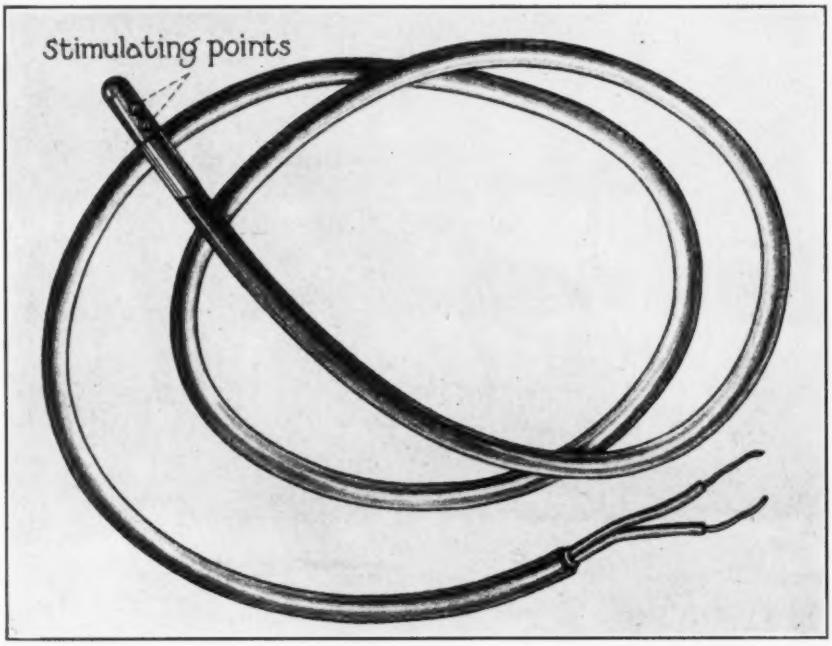

Fig. 2.—Special electrodes for intraesophageal stimulation. For description see text.

Fig. 2 shows a new form of stimulating electrodes made of two parallel wires insulated with a rubber covering about three-eighths of an inch in diameter. The wires, including the covering, are about as flexible as an ordinary stomach tube and are four and one-half feet long. At the stimulating end (hard rubber tip, water-tight connections) two small screws make contact with the inside wires. The flattened heads of these screws form the stimulating points of the electrodes. When the tip of these electrodes is passed down into the esophagus of an anesthetized dog and a stimulating current is applied, a variety of results may be obtained. If the electrodes are placed a little way above the cardia stimulation may inhibit breathing. If the electrodes be moved a little way, other nerve fibers may be caught which will cause vigorous and very rapid respiratory movements. Mixtures of these two effects may

also be obtained. Apparently they are due to reflex stimulation of the respiratory center. As the electrodes are drawn upward in the esophagus and new areas are stimulated, other phenomena occur, one of these being either slowing of the heart (possibly vagus inhibition), or the sudden development of auricular fibrillation. In most, but not all, dogs the auricular fibrillation can be produced readily. In some animals the fibrillation stops when the current is turned off, but in others it may persist for some minutes (Fig. 3). The type of anesthetic used undoubtedly influences the results. This is a good

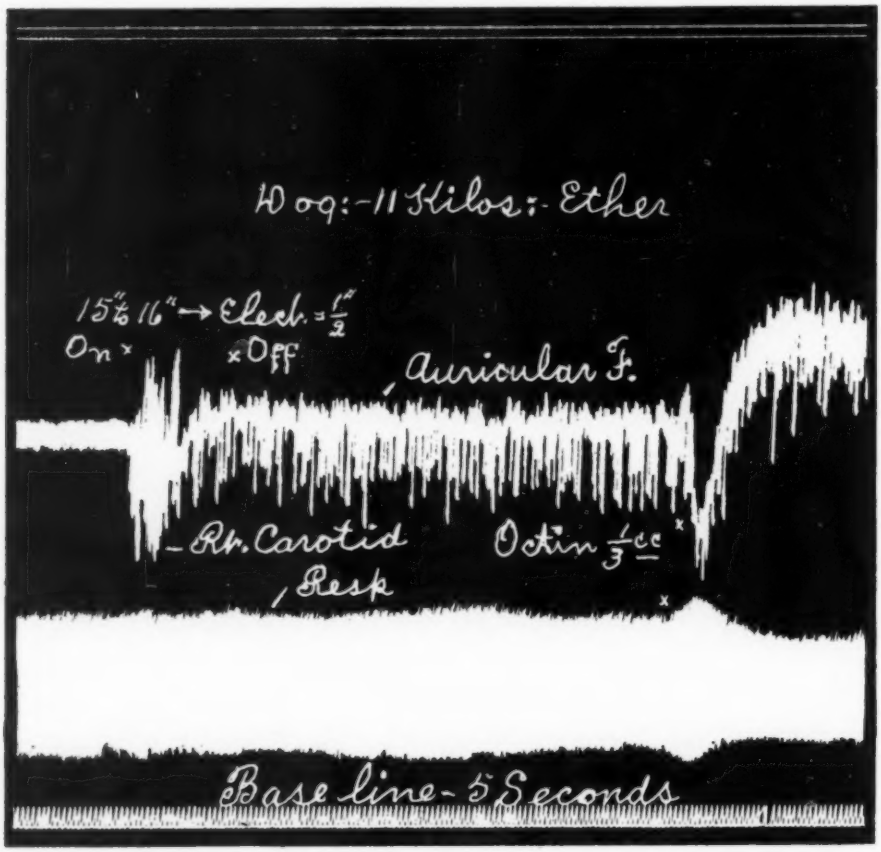

Fig. 3.—Upper tracing, blood pressure (carotid artery, mercury manometer). At "on" electrical stimulation was started within the esophagus directly behind the left auricle. The auricle promptly started to fibrillate and continued to do so throughout the rest of the tracing although the stimulation was discontinued at "off." Near the end of the tracing an injection of "octin" first lowered and then raised the blood pressure but did not stop fibrillation. Later an injection of adrenalin restored the normal beat.

way to produce auricular fibrillation for students who wish to study the phenomena in the intact animal. We believe that fibrillation is produced by leakage of current through the esophagus wall into the left auricle, but the sympathetic nerves in that area may be somewhat involved (as may some vagus fibers). We have not ventured to try this experiment in man for fear of the development of dangerous cardiac irregularities.

Stimulation within the lumen of the esophagus at most points above the level of the apex of the heart will produce vigorous muscular movements in some area of the chest, or in the fore limbs, neck, abdomen, or other parts of the body. This is due to stimulation within the esophagus of nerves which, in some way, transmit the impulses to the muscles of the involved areas. In the nonanesthetized dog weak stimulation thus applied moves exactly the same muscles (and apparently finer divisions of the musculature) and at the same time produces unmistakable symptoms of pain. The innervation appears to be *strictly unilateral* (and we believe this point to be of special importance particularly with reference to the injection treatment of angina). When the stimulating points are turned to the right, then movements (and pain if the animal is conscious) are produced in the right side. If the points are turned to the left, the movements (and pain) are on the left. But if the stimulation is toward the front or the back of the esophageal lumen, then movements may be obtained on both sides, apparently because the current spreads a little toward each side. With a little care one can pick out exactly the area into which pain spreads in angina pectoris (left arm, right arm, chest, etc.). We should emphasize here that these effects as seen in the *anesthetized dog* are mainly motor. But we have obtained a description of the muscular pains and soreness down the left arm of a patient only a few hours after he had had a severe attack of coronary thrombosis which involved, so far as it is possible for us to make comparisons between the human being and the dog, exactly the same muscles.

We should emphasize that by simply rotating the stimulating points from the left to the right within the esophagus we can produce exactly comparable movements on the right side. We believe that none of the involved nerves come from the true muscular tissues of the heart nor do they have anything to do with the heart directly (but the heart may be involved indirectly, or by reflex nervous action). For the strength of the heartbeat, the blood pressure, etc., may be entirely uninfluenced by the stimulation unless an area just beneath (behind) the auricle be stimulated (and then no movements of the limbs may occur). But if a few (efferent) sympathetic fibers going to the heart are caught in the stimulation, or if extra adrenalin is secreted, then there may be slight (delayed) changes in heart rate, etc.

Clinically it is well known that soreness of certain groups of muscles (usually down one arm) may follow attacks of angina pectoris or coronary thrombosis. Very rarely a herpes zoster follows down the course of the nerves in the arm and more often swelling, redness, other skin lesions or weakness occurs. We believe all of these symptoms result (directly or indirectly) from injury of these nerves in the esophagus wall or in the neighboring mediastinum and pleura. There is a good deal of clinical evidence to indicate that adhesions (from infections, etc.) between the esophagus and adjoining structures (pleura, bronchus, etc.) may be concerned in some cases. In such instances especially the longitudinal fibers of the esophagus (many muscular strands from the esophagus connect with neighboring structures) may produce a pull on nerve fibers which would be painful. Such endwise contrac-

tions of the esophagus can be seen if the outer wall of the organ is stimulated. These movements are of a rapid "creeping" character when produced by faradization. The esophagus seems to be capable of shifting its own position (also the effects of stimulation within it) somewhat from side to side (at least in the dog).

The difficulty of explaining both the presence and the absence of pain in the heart (coronary arteries?) during attacks of angina or coronary thrombosis has impressed many observers. And many literary (but fewer experimental) attempts have been made to clear the matter up. Within recent years von Bergmann,[2] Gionnoni and Lunedei,[3] and others have advanced the view that stimuli passing up the vagi from the stomach or esophagus go to the medulla and are thence reflexly sent back down over other vagus fibers (not having yet been felt by the patient) to the heart where constriction of the coronary vessels is produced, and this causes painful impulses which pass back through the posterior (anemia nerve?) roots to the spinal cord and thence to the brain when the patient becomes aware of their presence. We believe this conception is mainly erroneous, but partly correct. Von Bergmann has well described a whole series of patients (with x-rays) showing that "hiatus hernia" or dilatation of the esophagus at or just above the cardia is frequently found in patients having angina or "anginoid" symptoms. He has seen such a hernia which would balloon up suddenly just above the diaphragm when the patient breathed deeply. Many of these hernias are developed suddenly during fits of vomiting or straining. One of our patients dated her trouble definitely from the "breaking loose of something" deep behind the ensiform while she was attempting to place a heavy box on a high shelf. She died suddenly about four years later after having run a typical course of angina pectoris. We have found no x-ray pictures in the literature showing the condition of the stomach and esophagus *(with contained barium)* during an acute attack of angina pectoris or coronary thrombosis. But we know that vomiting is nearly always present in acute coronary thrombosis and sometimes in anginal attacks. The eructation of gas, or its retention in a relaxing (or contracting) stomach, of course, would not be seen by the patient or his attendants. Von Bergmann has expressed the opinion that there occurs an endwise (up and down) pull by the esophagus on the stomach, so that folds of the stomach may be drawn up through the esophageal orifice in the diaphragm (as shown in his x-ray pictures). This may be partly due to descent of the diaphragm in deep breathing which tends to compress the stomach.

"Hiatus hernias," diverticuli, dilatations, spasms and other anomalies or lesions of the esophagus in great number and variety are, of course, well known to gastroenterologists, but there is extremely little emphasis placed on these by cardiologists. And yet if one can get a true history of the exact location of the very first sensation which a patient feels in an attack of angina or coronary thrombosis, and if the physician can imagine that such an instrument as an ice pick were passed backward through the initial point of pain to the posterior wall of the body, it will very generally be found that the

instrument would pass through the esophagus or the upper part of the stomach. But secondary radiating pain in other regions may often be so severe that its point of origin is lost sight of entirely.

Especial mention should be made of the work of Walter Verdon[4] in England. Verdon believed that the real lesion of angina pectoris (he did not seem to recognize coronary thrombosis) consisted in the establishment of "irritable centers" in the spinal cord or medulla. He believed these "centers" were excited by stimuli that originated in the walls of the stomach or esophagus from pressure inside the organs which produced stretching (tension) on the gastric or esophageal outer coats, and that when these impulses reached the "irritable centers" the impulses were reflected outward over somatic nerves to the left arm, shoulder, neck, or other regions to which the pain was referred. A very large number of valuable clinical observations were made by Verdon. He did no animal experiments and does not mention the electrocardiograph, nor coronary thrombosis as we recognize it today.

If one opens the chest under anesthesia and records the pulmonary blood pressure from the left pulmonary artery, it will be found that inflation of a rubber balloon in the esophagus just behind the heart will cause an enormous rise in the pulmonary blood pressure (Fig. 4). A similar inflation of the esophagus by entrapped air (or stomach contents), due to two approaching spasmodic contraction rings, may very well be expected to produce a comparable physiologic effect with increase of pressure in the right heart (causing, as can be shown experimentally, axis deviation, T-wave changes, etc.), dyspnea, dysphagia or anginal pain. It has been fully demonstrated by Hertz[5] and by Payne and Poulton[6] and by others that rapid inflation of a balloon in the esophagus (even with low pressures) causes the development of pain which may easily be made so acute that the subject cannot stand it, and that the subject can locate the position of the pain (bag), as revealed by fluoroscopy, within one inch of its exact location. It is our opinion that a patient who indicates over the left border of his sternum, or over the ensiform, a spot which marks the seat of his pain deep down in the chest is actually locating within one inch the exact origin of his pain in the esophagus or neighboring tissues. If the pain radiates down one or both arms, or into the chest or back, then we believe that the esophageal dilatation (or endwise traction) has involved the nerves (in the esophageal wall, mediastinum, pericardium or pleura) which we have discussed above with reference to intraesophageal electrical stimulation.

When a bag is inflated in the esophagus behind the heart, the presence of the bag may impede or close off passage of blood through the pulmonary veins from the lungs back to the left auricle. This may tend to lower the aortic pressure, but to cause engorgement (possibly painful) and cyanosis on the systemic venous side. The position of the bag (or dilatation) up or down the esophagus will vary the results obtained. We believe that this phenomenon is most likely the cause of so-called "cardiac asthma" (Fig. 4). But there are at least two other possibilities which we have considered. These are the possibility that the dilatation may mechanically compress the bronchi (or

auricle), or that dilatation (possibly longitudinal contraction) of the esophagus may stimulate respiratory-inhibitory nerves in the esophagus as we have above indicated (by electrical stimulation in our experiments). A cross-section of the chest at the level of the base of the heart will show that the esophagus lies directly behind the heart and that the pulmonary veins (posterior—low or negative pressure) are most likely to be compressed before the pulmonary arteries are affected. Incidentally, in passing, it may be noted that a pain in the base of the heart or one in the adjacent esophagus might be only about a quarter

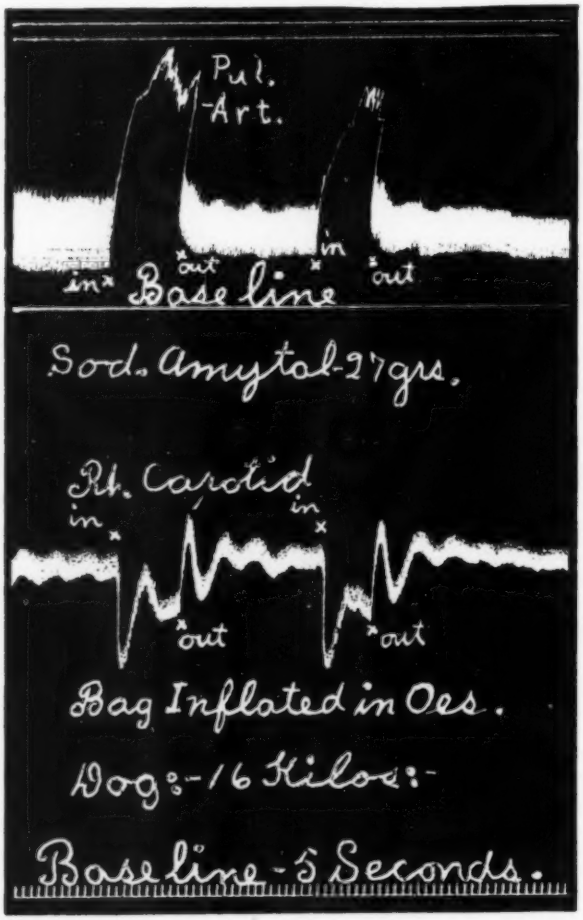

Fig. 4.—Upper tracing, pulmonary blood pressure; lower tracing, carotid blood pressure. Inflation of a rubber balloon (twice) in the esophagus behind the heart (compressing the pulmonary veins and left auricle) blocked the return flow of blood from the lungs to the heart and greatly raised the pulmonary pressure but tended to lower the systemic pressure. For discussion see text.

of an inch apart, and it would be quite impossible for the patient, let alone the heart specialist, to distinguish between these locations on the basis of the pain alone. And any sensory nerves coming from the heart would pass through this region, and hence would be subject to injury at this point.

Experimentally in dogs and fluoroscopically in man, it can be shown that the esophagus may frequently undergo peristaltic movements both upward and

downward (Fig. 5), especially during vomiting, retching, and similar states. For this experiment it is advisable to fill the esophageal bag with water rather than air. The local nervous mechanism[7] seems capable of carrying on these movements independently of the vagi and sympathetics, but no doubt these nerves always exercise a control under normal conditions. But under strong emotional stress vagus action undoubtedly can influence these peristaltic movements both in the esophagus and in the stomach. This we believe to be the chief etiology of "nervous indigestion" and of emotional anginal attacks.

A full stomach, or one containing *much gas,* we believe to be the usual initiating cause of an acute attack of angina pectoris, or of the commonly

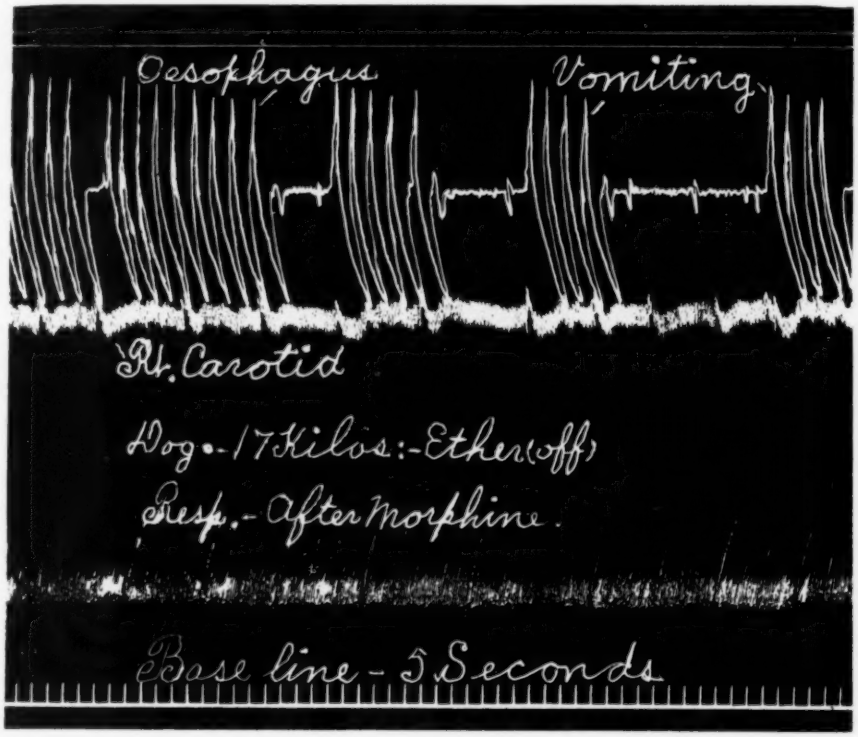

Fig. 5.—Upper tracing, esophagus; middle tracing, blood pressure; lower tracing, respiration. Esophagus tracing obtained by placing a rubber bag filled with water in the esophagus just behind the heart. Vomiting, retching, nausea, etc., produce slowly moving peristaltic contractions in the esophagus. In man similar movements are carried out and the subject is often entirely unconscious of them. We believe such movements as these (exaggerated) and also longitudinal movements pull on nerves or other structures in the mediastinum to produce the pain of angina (and coronary thrombosis).

recognized form of coronary thrombosis. A high strung, nervous individual whose stomach becomes upset following a sudden death in the family (we now have two such patients under observation), or following any other incident which greatly excites his emotions, is liable to be seized by an anginal, or "anginoid" attack (perhaps the first he has ever experienced). We believe all of these attacks to be of essentially similar character and etiology but to vary in degree and in the location of the pain (which often is not pain but rather dyspnea, see Fig. 4). These results may be brought about in at least

three different ways. First, exercise leads to increased breathing and hence increased compression of the stomach by the diaphragm and abdominal muscles. Von Bergmann's x-ray plate shows a patient with a hiatus hernia into the esophagus which could be seen to fill up suddenly when a deep breath was taken. The presence of such foreign material in the esophagus, and under increasing pressure from the stomach, will be very likely to stir up spasmodic, incoordinated contractions in the esophagus. In stimulating the lumen of the lower end of the esophagus in nonanesthetized dogs with a weak current, we have usually produced vomiting, among other phenomena. And vomiting in dogs is associated with irregular, peristaltic movements of the esophagus (see Fig. 5). And Hertz, and Payne and Poulton have shown clearly that pressure (especially sudden) inside the esophagus produces pain which increases in intensity with the pressure, and which the patient can accurately locate. We cannot be sure but that the longitudinal muscle layers of the esophagus (especially in the presence of pleural adhesions) may cause some of these results. And a further point to be emphasized is the increased intermittent pulling on the lower end of the esophagus (smooth muscle tonically resists tension) which deep breathing from exercise may bring about, especially with a full stomach.

Second, we believe an "overflow" of nervous impulses along the vagi to the stomach and esophagus may stir up abnormal contractions, and in a susceptible patient these contractions may become incoordinated and thus entrap air or other stomach contents in the stomach or esophagus or both. This is the "emotional" type of attack (sometimes cured by psychotherapy), and it is directly related to "nervous indigestion" of which the patient may or may not complain. It must be dependent on anatomical abnormalities in the stomach or esophagus (or adhesions of the esophagus), or to derangement of the local nervous mechanism in the walls of the esophagus or stomach, or to a central derangement of the impulses which pass down the vagus (possibly the sympathetics) to the esophagus and stomach. The stomach wall contains only smooth muscle while the lower third of the esophagus is smooth muscle, the middle third mixed, and the upper third is entirely striated. But the entire length of the esophagus is innervated (vagi and sympathetics) in exactly the same fashion as an ordinary smooth muscle structure. Hence, we would not be surprised to find occasional anomalies in esophageal contractions, especially in the middle third, and these would be likely to extend to other parts of the esophagus. We have collected a good deal of data which make us suspect that some individuals have a fair amount of voluntary (but unrecognized) control over certain esophageal contractions. For example, we had a patient who could "fight off" anginal attacks quite often. We believe that was done through the vagi by some form of inhibition, or recoordination, of esophageal or stomach contractions. There are other such cases indicated in the literature. (We consider here, of course, that the coronary arteries have nothing whatever to do with angina, but angina may often secondarily involve the heart.)

Third, attacks of angina may be induced directly by indigestion. These are more likely than the other types to lead to acute coronary thrombosis and frequently are fatal, either within a day, or within two to four weeks. But

partial recovery with later attacks may frequently occur. These patients may be hale and hearty, are usually good eaters, often fair drinkers, and may or may not have previously had "indigestion." The Cincinnati papers usually carry notices of four or five of these cases every day. Attacks may come on at any time, often in sleep. If the patient makes a good recovery with no observable lingering after-effects, he is said to have had indigestion (Fig. 6).

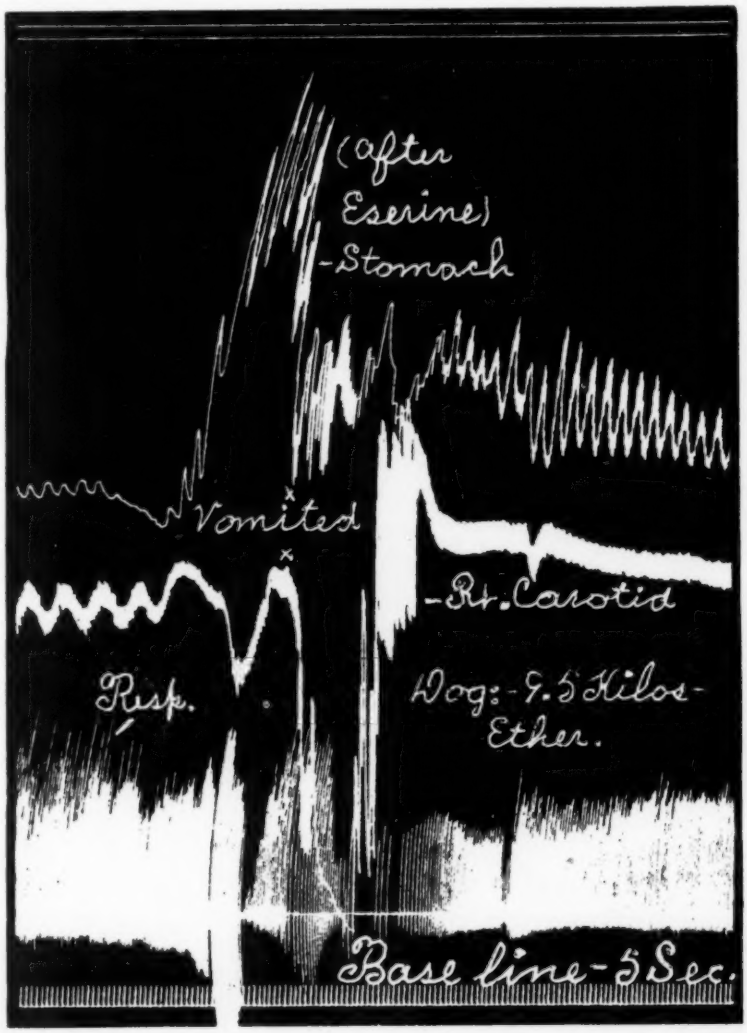

Fig. 6.—Upper tracing, stomach; middle tracing, blood pressure; lower tracing, respiration. A very small amount of eserine had been given to partly overcome the ether depression so that stomach contractions could be obtained. Following a large stomach contraction the animal vomited at "X." There was some respiratory difficulty and then the heart was strongly inhibited (reflexly) and finally stopped for half a minute or more. The heart finally broke through the inhibition and returned to normal. Such excessive inhibition is apparently the cause of death in many cases of angina pectoris.

But if he gets a severe painful esophageal or mediastinal injury, with edema around the roots of the lungs, râles, slight fever, some leucocytosis, perhaps with distant heart sounds and a pericardial friction rub (probably from dis-

placement of the heart by mediastinal swelling), possibly electrocardiographic changes or fall in blood pressure, or, especially if he dies within a few hours, then the diagnosis is coronary thrombosis. But if he dies suddenly in the early stages of the attack, then it is angina pectoris (see Fig. 6). At the autopsy the coronaries may be found to be entirely normal with little or no visible pathology in the heart. Or an actual thrombosis may be present. We believe that the pain in these cases is not due to the coronary thrombosis per se, but to esophageal or mediastinal injury (the stomach may be involved in some cases, i.e., in the region of the cardia). The pain from an actual rupture of the tissues with absorption, etc., will, of course, be likely to last for at least some hours.

Why sudden death in these cases? Fig. 6 illustrates well what is probably the most common cause, i.e., excessive vagus inhibition from reflex stimulation of (sensory) vagus fibers by stretching or injury of the esophageal wall (the stomach may be equally involved) in the act of vomiting or by entrapment of gas (without vomiting). When no thrombi are present in the coronaries, i.e., when the chief initating cause of death lies *outside* the heart, we believe from both clinical and experimental evidence that inhibition is the most probable cause of death. But when the coronaries are thrombosed (i.e., the chief initating cause of death may be *within* the heart), then we suspect that ventricular fibrillation will be the most likely cause of sudden exitus. Slow death from weakening of the heart and general exhaustion may, of course, occur in these cases.

It is interesting to note that the first case of coronary occlusion ever diagnosed during life (by Adam Hammer[8] in St. Louis in 1876) occurred in a patient who had *no pain whatever* during his attack, which lasted some thirty hours, and was due to the formation on an aortic valve of a thrombus which extended up and occluded the right coronary artery. The total absence of pain in this case (and there are many others in the literature now, Herrick,[9] Davis,[10] Levine[11]) convinces us that anemia of the heart per se (from constriction of the coronaries or anything else) does not produce the pain of angina pectoris. And there is an abundance of other evidence, both experimental and clinical, to prove this point, which (together with other vague notions of pain in the *heart*) has hampered medical progress with respect to angina pectoris ever since the suggestion involving the coronary arteries was first surmised by Jenner. And the suggestion that angina pectoris was like intermittent claudication, in our opinion, only made bad matters worse.

Drugs.—Nitrites we believe exercise their beneficial action by relaxing the stomach and esophagus. We have personally felt a relaxation of the pyloric end of the stomach by nitroglycerine which promptly relieved colicky pains which we knew to be due to gas in the stomach. This is obviously their action in seasickness. Theophylline and its congeners may afford some relief by their diuretic action, but there is some evidence that they may exercise a very slight sympathetic (adrenalin-like) action, and we believe that such slight benefit as they may afford is due to this action on the stomach and esophagus. Digitalis irritates the stomach and is liable to make angina cases worse, but the

drug may be indicated for coincident cardiac conditions. Morphine may relieve the pain of angina pectoris not only by its central action but also by relaxing[12] the stomach walls. Papaverine acts similarly on the stomach (not centrally). Atropine relaxes the stomach, but the required dose is probably too large to be of use clinically. Adrenalin relaxes the stomach and (to a less extent) the esophagus, but its central and other actions (fear, excitement) are liable to induce attacks. It dilates the coronary arteries.[13] Administration by mouth (slow and imperfect absorption) may be helpful in certain cases. Trichlorethylene has been used by Krantz. It acts centrally as an anesthetic. Insulin, by indirectly increasing the (hunger) contractions of the stomach, may make the attacks worse.

Within recent years much brilliant surgical work has been done on angina pectoris. In our opinion the chief conclusion to be drawn from all this work is that it has added further evidence to show that the coronary artery explanation of angina pectoris is wrong, and that the explanation we have offered above is correct.

Finally, it has been shown by Verdon[14] "on many occasions and always with satisfactory results" that during an acute attack of angina pectoris passage of a stomach tube through the esophagus into the stomach has led to prompt elimination of a large volume of gas, and sometimes of fluid contents of the stomach, and to the immediate, usually complete, relief of the patient. We believe that practically every conclusion regarding this disease that has heretofore been reached on a basis primarily of pathology alone is wrong. For, in our opinion, angina pectoris is an affliction of the living only, not a state of the dead.

CONCLUSIONS

1. We have shown that electrical stimulation inside the esophagus at appropriate locations within the chest produces muscular contractions and pain in exactly those areas of the body in which pain (and later soreness) is developed during acute attacks of angina pectoris and coronary thrombosis. The innervation we believe is strictly ipselateral and does not come from the heart.

2. We believe that the coronary artery spasm explanation of angina pectoris is erroneous.

3. We believe that angina pectoris is due to acute incoordinated spasmodic contractions of the esophagus (including its longitudinal muscle layers) and stomach whereby gas or other stomach contents are entrapped under pressure, and the walls of either viscus with their contained or adjacent nerves and tissues are strained or injured. We have presented a small part of our extensive experimental and clinical evidence to support these views, and we have referred briefly to coronary thrombosis.

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COMPLETE TEMPORARY RECOVERY, OF LONG DURATION, IN ACUTE ALEUCEMIC MYELOID LEUCEMIA*

A Case Report

Israel H. Marcus, M.D., Brooklyn, N. Y.

RECOVERY, even temporary, with *complete* return to normal of the symptomatology, physical signs, and especially the blood picture, is so rare in myeloid leucemia, that the following case is reported in detail.

C. N., a Hebrew housewife, fifty-eight years of age, entered the hospital on May 5, 1930, having suffered for nine weeks with anorexia, lassitude, increasing pallor, fever of 100.5° to 103.5° F., pain and swelling of the knees, elbows, ankles, and wrists, the joint symptoms persisting for a day or two in one joint and then traveling to another. During the last four weeks there had appeared at separate occasions on the left forearm two vesicles, containing serosanguineous fluid, upon ecchymotic bases and surrounded by induration and erythema. Each had persisted for about a week.

Family History.—One sister has Hodgkin's and one brother has Vaquez's disease.

Previous History.—Unimportant except for pneumonia in 1918.

Physical Examination on Admission.—Temperature 103° F. Marked pallor of both skin and mucous membranes. *Mouth:* The tonsils were buried and cryptic, and much foul smelling discharge could be expressed from the left one. *Chest:* No sternal tenderness. *Lungs:* There was dullness, decreased breath sounds, and a few large moist râles at both bases. *Heart:* Slightly enlarged to left. There was a soft systolic apical blow. A_2 was louder than P_2. Blood pressure 130/80. *Abdomen:* Liver edge was felt 2 fingerbreadths below

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the costal margin. Tip of spleen was barely felt. *Extremities:* No edema. The knee joints, elbows, and left wrist were painful on active and passive motion, and were tender. Both knee joints were swollen. No redness of the joints.

Laboratory Findings.—Blood Wassermann and Kahn tests, blood cultures, blood Widal, and blood chemistry examinations revealed no abnormalities.

X-Ray of the Chest on Admission.—Negative.

Progress of the Patient.—Shortly after admission a blood study showed 2,500,000 R.B.C., with 36 per cent hemoglobin, 6,400 W.B.C. with 14 per cent neutrophilic polys, of which 9 were band forms and 5 segmented, 40 per cent lymphocytes, 24 per cent myeloblasts, and 22 per cent monocytes, and only 30,000 platelets. The hematologist believed that the cells he designated monocytes were not true monocytes but were rather forms of myeloblasts. He thought the condition was a true acute myeloblastic leucemia. The blood study, like all the others done on this patient, was made by one of a group of physicians who had been specializing in hematology for a number of years. On the day after admission pinpoint hemorrhages were seen in the left conjunctiva and large hemorrhages in both eyegrounds. About a week after admission the joint trouble had become markedly ameliorated. Two weeks after admission there was noted a nontender, somewhat bluish infiltration in the subcutaneous tissue of the right axilla, not involving the lymph nodes. There were at this time for a few days on the buccal mucosa small spots of whitish exudate, which, when rubbed off, showed a reddened base. The patient was treated with blood transfusions, liver extract, ventriculin, sodium cacodylate subcutaneously, and large doses of iron by mouth. After June 16 the temperature, which had been at 101° to 104° remained at or below 100°. By June 19 the skin infiltration in the axilla had completely disappeared, the liver and spleen could no longer be felt, and the lungs and heart appeared normal. Meanwhile the patient had grown much stronger, and her blood status had markedly improved, though a marked increase in the lymphocytes persisted for a long time. Thus in a study on June 30 she had 4,000,000 R.B.C. and 67 per cent hemoglobin, but there were still 62 per cent lymphocytes. On July 22 a blood study showed 4,300,000 R.B.C., 84 per cent hemoglobin, 47 per cent lymphocytes, 47 per cent neutrophilic polys with 4 staff forms, 3 per cent eosinophiles, 3 per cent monocytes, and 250,000 platelets. The patient was discharged on July 26, 1930.

At home the patient was able to do her usual household duties. On Sept. 10, 1930, a blood study showed 6,000,000 R.B.C., 82 per cent hemoglobin, 11,200 W.B.C., with 58 per cent neutrophilic polys, ½ per cent eosinophiles, only 38 per cent lymphocytes, 3½ per cent monocytes, 0.1 per cent reticulation, and 240,000 platelets. No abnormal cells were seen.

On May 9, 1931, i.e., about ten months after her discharge from the hospital, the patient was seen complaining of a cough of two months' duration, especially bad for the last few days, anorexia for two months, and weakness for several weeks. Examination showed the patient to have a temperature varying between 100° and 103° each day. She did not appear acutely ill. The pharynx was congested, the tonsils cryptic. Lung examination was negative except for rare râles scattered throughout. The heart examination was negative. Blood pressure 135/90. The edge of the liver and tip of the spleen could now again be barely felt. There was no edema. The superficial lymph glands were not enlarged.

On June 5 many small purpuric spots, each 1 to 2 mm. in diameter, appeared on the skin of the abdomen and back. The patient was meanwhile growing markedly weaker, and on June 10 she was very dyspneic. Pallor was now marked. On the hard palate and buccal mucosa were several small ecchymotic areas and a small ulcerated area covered with a thick exudate. Her face was full and puffy. At the right lung base there were now heard numerous crepitant râles and diminished breathing. The abdomen was distended, and shifting dullness was present. X-ray of the chest showed a definite infiltration at the right base with an overlying haze. On June 11, the patient became irrational, the temperature rose to 104.8°, and she died early the next day.

Laboratory Studies.—Blood chemistry on admission and two days before death: Normal. Blood culture: Sterile. On June 10 the urine, which had previously been normal, now showed 2-plus albumin, many white blood cells, a rare red blood cell, but no casts.

Blood Studies.—As is seen in the chart the white blood cells, which on May 14, 1931, were only 8,200, on May 27 were 20,400, and on June 2, 38,200. The polys were constantly

DATE	MAY 6, 1930	MAY 11, 1930	MAY 16, 1930	MAY 26, 1930	JUNE 3, 1930	JUNE 6, 1930	JUNE 9, 1930	JUNE 19, 1930	JUNE 30, 1930	JULY 12, 1930	JULY 22, 1930	SEPT. 10, 1930	MAY 14, 1931	MAY 16, 1931	MAY 19, 1931	MAY 27, 1931	JUNE 2, 1931	JUNE 4, 1931
Hemoglobin, %	34	36	38			44		60	67		84	82	48	51	62	61	46	46
Red blood cells in millions	1.2	2.5	2.7			2.9		3.4	4.0		4.3	6	2.9	4	3.9	3.3	2.7	2.5
White blood cells	4,700	6,400	5,600			3,800		4,200	7,800		10,200	11,200	8,200	7,300	7,200	20,400	38,200	33,600
Neut. Polys., %	20	9-B 5-S	14	19	27	36	44	33	33	44	4-B 43-S	4-B 54-S	4-B 6-S	4-B 6-S	4-B 4-S	2-B 2-S	2	3-B 2-S
Lymphocytes, %	44	40	47	64	63	61	50	63	62	49	47	38	66	50	52	37	37	20
Myeloblasts, %		24			⅓		1							1	3	5	60	10
Monocytes, %	35	22	38	6	6	1	3	1.6	2	4 5	3	3.5	22	38	34	50		65
Eosinophiles, %	1			2	1.7	1	2	1.6	2	3	3	0.5						
Myelocytes, %			1	3	⅓		½								¼			
Oligochromasia			Sl.	Mod.			Mod.		Sl.					0		0		
Polychromasia			Sl.	None		Mod.	Mod.	Mod.	Sl.				0	Sl.	Sl.	Sl.	Sl.	
Stippling			2/400		2/300	1/200			1/200					1/400	2/400	0	0	
Poikilocytosis			Sl.			Sl.	Mod.						Sl.	0		0	Mod.	
Reticulation, %	0.5		0.5	3	2	4	3	4		0.8		0.1	0.5	1	0.5	1	1	
Normoblasts	1/300		3/400	0	1/300	2/200	1/200	1/200					1/500	2/400	1/400	7/400	8/400	
Macrocytosis	Very Rare			Sl.		Sl.	Sl.	Mod.	Very Sl.					0		0	0	
Anisocytosis	Very Sl.		Sl.			Mod.	Mod.	Mod.	Sl.				Sl.	0		Sl.	Sl.	
Microcytosis						Sl.	Mod.	Sl.	Very Sl.					0		0	0	
Turk cells, %					1.7			1	1									
Basophiles, %									0.5									
Metamyelocytes, %					⅔													
Hyperchromia								+	+	Sl.								
Peroxidase Neg., %			70						M-59								M-74	
Peroxidase Pos., %			30						P-38 M-3								P-3 M-23	
Rieder cells, %																		
Plasma cells													0.8	0.5				
Lymphoblasts													0.4		0.8	0.8		
Histocytes													0.8		2	2.3		
Megaloblasts, %						1/200								1/400				
Coagul. time (min.)	2		5			5			5 ½			8	1/500	0	2/400	1/400	1/600	
Bleeding time (min.)	4		1 ½			4			1			2 ¾	8	6				
Color index	1.5	0.7	0.7			0.8		0.9	0.8		1	0.7	3	1			3	
Platelets (in thousands)	120	30	90			120			220		250	240	0.8	0.7	0.8	0.9	0.8	0.9
Examiner	A	B	C	D	C	C	C	C	C	C	B	C	80 C	70 C	100 C	150 C	150 C	70 B
Remarks		The monocytes are forms of myeloblasts.	15 of the monocytes resemble atypical myeloblasts.	The monocytes are myeloid monocytes.						No abnormalities of red blood cells.	No abnormal cells.	No abnormal cells.	Not certain whether the 22 are monocytes or pathologic myeloblasts.	Not certain whether the 38 are monocytes or pathologic myeloblasts.	Not certain whether the 34 are monocytes or pathologic myeloblasts.	Not certain whether the 50 are monocytes or pathologic myeloblasts.	Many of the myeloblasts are atypical.	The 65 are altered myeloblasts and show a negative oxydase reaction.

S, Segmented forms.
B, Band forms.
Sl., Slight.
Mod., Moderate.
1/300, 1 seen while counting 300 white blood cells.
P, Polymorphonuclears.
M, Mononuclears.

but few in number. The lymphocytes at first were as much as 66 per cent, but decreased later to 10 per cent. Many large monocytic cells were seen, which the hematologist, at first not sure whether or not they were atypical myeloblasts, later decided definitely were myeloblasts rather than true monocytes.

COMMENT

It is felt that the findings in this case were so indicative of true myeloid leucemia that the diagnosis was justified even though an autopsy could not be secured. In Naegli's clinic, too,[1] it is believed that the diagnosis of leucemia can be made on the findings during life in some cases, without postmortem examination.

The difficulty encountered in classifying the large mononuclear cells of this patient's blood as monocytes or myeloblasts illustrates a difficulty frequently met with in acute leucemia.[2]

The case demonstrates the danger of considering a patient with leucemia as permanently cured until a very long time has elapsed without a recurrence of the disease. Pearce[3] reports a cure, although the period of observation after recovery was not four months.

SUMMARY

A case of aleucemic myeloid leucemia is reported, which at the outset appeared to be of an acute type. The patient, however, made a complete recovery for eight to ten months, with no evidence of the disease either on physical examination or blood study, and then again evidenced the findings of acute myeloid leucemia and died.

The case is of further interest because of the following: (1) A family history of having a brother suffering from Vaquez's disease and a sister with Hodgkin's disease, (2) the axillary skin infiltration which later disappeared, (3) the occurrence of vesicles on the skin containing serosanguineous fluid with induration about the vesicles, (4) the onset of the disease with joint manifestations strongly suggesting rheumatic fever, (5) only slight splenic enlargement at any time, and (6) the comparatively large number of lymphocytes found at various stages throughout the disease.

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COMPARISON OF THE XYLOSE TOLERANCE WITH BLOOD UREA IN NEPHRITIC RATS*

HARDY W. LARSON, PH.D., NEW YORK, N. Y.

THE clearance of xylose,[1] used as a measure of renal function, is believed to give results comparable with the urea clearance. Folin[2] was of the opinion that renal injury should be reflected in a slight retention of urea which would be recognizable, provided the range of normal urea was sufficiently established. He believed that a simple determination of blood nonprotein nitrogen might prove of value in detecting early kidney damage. Peters and Van Slyke,[3] however, hold that such a determination is of no value for early diagnosis, and that blood nonprotein nitrogen does not rise until renal impairment is far advanced. The latter view is generally accepted. This study was planned to obtain information on these points.

Because of the extremely low threshold of the kidney for xylose, Fishberg and Friedfeld[4, 5] advocate the use of this pentose as a delicate index of damaged renal function. They found that after the ingestion of 50 gm. of xylose on a fasting stomach and with limited fluid intake, the blood nonfermentable reducing substances rose to a maximum within three hours, to return to a value approaching the original fasting figure within five hours. If the kidney function was impaired, this five-hour value remained high, and a considerably longer period, depending on the amount of kidney damage, was required before the xylose was removed from the blood stream. In the present investigation animals with progressive nephritis were used to study the points involved.

EXPERIMENTAL

Young rats approximately six weeks old were used for the experiment. One pole of the kidney was ligated to cause degeneration beyond the ligature, and two weeks later the other kidney was removed. The rats were then placed on a diet containing 43 per cent dried liver which was known to produce renal injury. A month later the animals were given xylose. They fasted overnight and were then given 0.1 gm. of the sugar per 100 gm. body weight by medicine dropper, and the nonfermentable sugar of the blood followed for five hours. The rats were bled before xylose was given and one and one-half hours and five hours after the pentose was administered, the blood being obtained from the tail. Two-tenths cubic centimeter of blood was laked in 3.8 c.c. H_2O in a centrifuge tube. One cubic centimeter of a 25 per cent yeast suspension, which had been washed three times, was then added, and the tubes were allowed to stand one-half hour at room temperature. Five cubic centimeters tungstic acid made from equal parts of H_2SO_4 (120 c.c. of 0.666N H_2SO_4 in 1 liter) and

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sodium tungstate (120 c.c. of 10 per cent $Na_2WO_4.2H_2O$ in 1 liter) were then added, and the tube centrifuged. Five cubic centimeters of the tungstic acid filtrate were used for the nonfermentable sugar determination by the Folin and Malmros[6] micromethod. Urea nitrogen was determined on 0.2 c.c. of blood by the manometric method of Van Slyke[7] for whole blood. The urine was collected from the metabolism cages during the fasting period; it was tested for albumin and the sediment examined. This procedure was repeated at monthly intervals until either the xylose curve became abnormal or there was retention of urea. The rats were then killed and the kidneys and livers examined for pathologic changes. Approximately half of the number of kidneys appeared to be normal on histologic examination. The other 50 per cent showed pathologic evidence of early nephritis. The ligature itself did not seem to cause hastening of the process, even in cases where half of the kidney had atrophied. Males were more prone to develop nephritis than females. In the group reported as nephritic, 14 of the 15 were males. In the nonnephritic group, the sexes were about evenly divided. The large majority of rat livers showed albuminous degeneration, and the liver cells appeared swollen.

The results obtained with xylose are very difficult to interpret. The xylose curves are very erratic and show wide discrepancies, and there is no apparent correlation between xylose clearance and urea retention. Approximately half of the rats exhibited xylose clearances which varied considerably from month to month. At times the final value would be back to the fasting level in five hours. The next month it would be high, the following month low, etc. As this test is supposed to be a delicate index of renal function, the conclusion would be that these rats were nephritic one month and normal the next. Certainly there is no evidence of the progressively greater xylose retention from month to month which would be expected if we assume that kidney injury was becoming more pronounced. Some rats showed apparently normal xylose curves for several months before there was any elevation of xylose, although increased values for blood urea and the appearance of casts and large amounts of albumin in the urine indicated much earlier kidney damage. On the whole, the monthly urea values were more consistent than the corresponding xylose figures. Although there were exceptions, the urea values showed less tendency to fluctuate widely, and there was more likely to be a small monthly rise. This is in keeping with the assumed progression of the kidney impairment.

In Fig. 1 the rats are divided into two groups. In the experimental group are those which had one kidney removed and the pole of the other kidney ligated, and which were fed a high protein diet. In this group the rats are classified as nephritic or nonnephritic, according to the pathologic findings. In the other group are the normal or control rats which were not operated upon and which were fed a regular stock diet. The final xylose figure for the fifth hour is plotted as the vertical line. The small circle on each of these lines represents the corresponding urea value for this final determination.

It is seen at a glance that the xylose and urea values do not correspond. There may be high xylose with low urea and vice versa. The average xylose figure for 14 rats showing no evidence of nephritis, and having apparently normal kidneys, is 27.1 as compared with 33.3, the average for 15 rats with

pathologic kidney. It is seen that in the minimum nephritic value and the average for the nonnephritic are approximately the same, as are the nephritic average and the maximum nonnephritic. In the group showing evidence of nephritis on pathologic section, 9 of the 15 xylose values are above the maximum

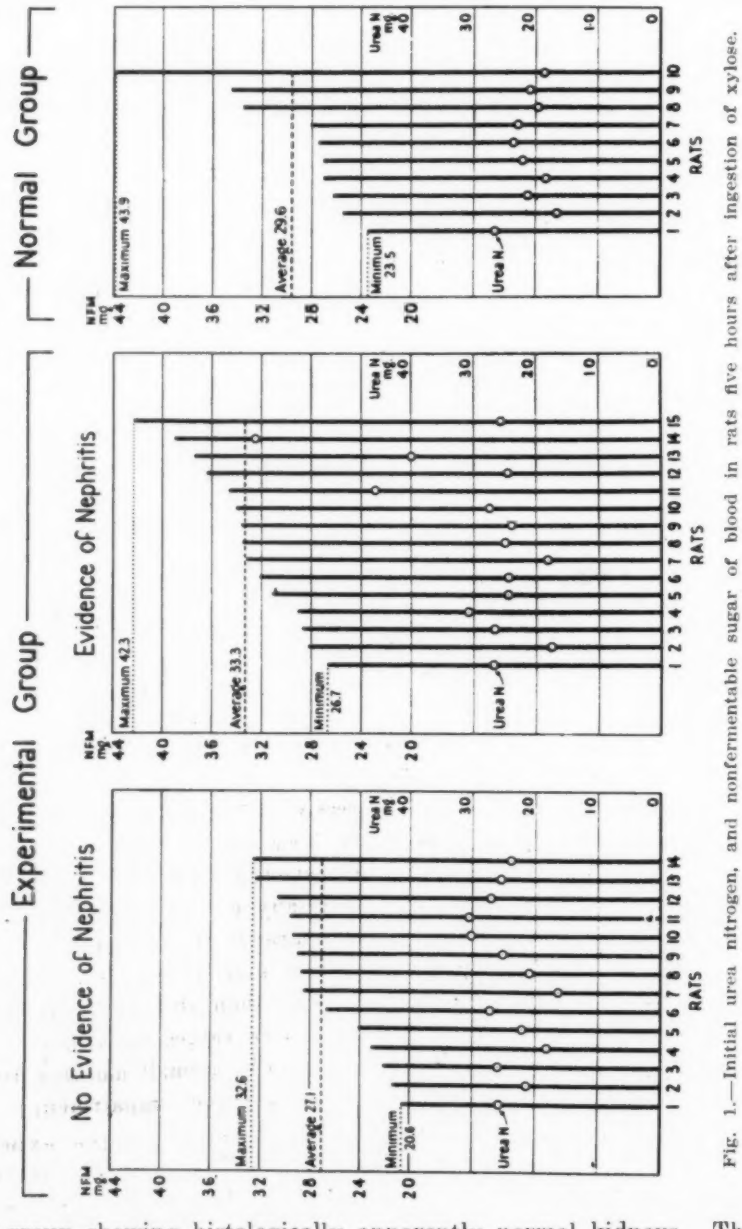

Fig. 1.—Initial urea nitrogen, and nonfermentable sugar of blood in rats five hours after ingestion of xylose.

for the group showing histologically apparently normal kidneys. This might be interpreted as showing a definite and positive trend toward higher xylose values in rats in which renal injury can be demonstrated by pathologic technic. Ten of the 14 rats in which no histologic evidence of nephritis is found show xylose values lying approximately between the minimum for the group in

which nephritis is shown and the average for this group. Where there is no evidence of histologic change in the kidney, there may still be a functional change not detectable by histologic methods. It may be that xylose clearance and urea retention will indicate renal injury before any histologic change can be demonstrated.

In the nephritic group, Rat 15 shows the highest xylose value, yet pathologically Rats 13 and 14 show a greater degree of renal damage. Likewise, they exhibit much higher urea values, and their urines contain more albumin and a greater number of casts. From this it would appear that they were more nephritic than Rat 15, but the xylose figures do not show it. As the xylose test is assumed to be a delicate indicator of renal function, it would seem that the highest xylose values should be obtained in those rats exhibiting the greatest degree of renal damage pathologically, the most urea and albumin, and which give evidence of functional derangement by the greatest number of casts. Such, however, is not the case.

Although there is a distinct trend toward higher xylose values in the nephritic group as compared with the nonnephritic, the xylose test does not appear as favorably when applied to a group of normal rats. This group of rats was not operated upon and was fed a regular stock diet. They were apparently normal in every respect except in their reaction to the xylose test. Histologically they showed no evidence of nephritis. The urea values were low and the urines contained neither casts nor large amounts of albumin. In spite of this, the xylose figures are not low, as would be expected. They cover a wide range, the average of which is higher than that of the nonnephritic group, and there is a higher xylose value in this normal group than any obtained in the nephritic group. Thirty per cent of the xylose values for this normal group are above the pathologic average. In many cases in this group of normal rats, the results obtained with xylose would lead one to believe that one month some of the rats were definitely nephritic, and the next month normal, etc., because the xylose values were high at times and at other times were back to the fasting level.

Because of the wide variation in the control values, it was felt that this work should be repeated, using a larger number of animals. Twenty young rats of approximately the same age as those used in the control group were given xylose and the blood nonfermentable sugar followed for five hours. Four of this group showed final xylose values which were high. The clearance was repeated at the end of two weeks. Again four final xylose values were high, but only one rat which showed a high xylose in the first clearance was included in this group. The seven rats which gave high values in either the first or second clearance were again given xylose. Six of the seven again showed high xylose values. Only one of the seven gave a high final figure in all three clearances. This again demonstrates the variability of the xylose clearance in normal rats.

The most important finding in this work is the fact that albumin and casts appear in the urine several months before there is retention of urea or the xylose curve becomes abnormal or there is definite histologic change. The presence of albumin and casts indicates a functional disturbance of the kidney which occurs much earlier than the anatomic changes characteristic of nephritis.

In Protocol I, a representative case is taken. The kidney of this rat was reported as showing slight early nephritis. The blood urea remained at a fairly low level for five months, but the albumin increased from 100 to 600 mg. per 100 c.c. within the first month, and the urine sediment showed several casts and cylindroids at the end of the second month. The first xylose curve shows the fifth-hour value approximately down to the fasting normal. The second month shows an elevation. The third, fourth, and fifth months show apparently normal curves. The fifth month shows the fifth-hour xylose value down to the fasting figure at the time 47 granular casts and 40 cylindroids, and an albumin of 800 mg. per 100 c.c. were reported.

PROTOCOL I

EXPERIMENTAL FINDINGS WITH RAT 773 ♂. SLIGHT EARLY NEPHRITIS

DATE	BLOOD		URINE	
	XYLOSE NFM	UREA N	ALBUMIN	SEDIMENT
	MG. PER 100 C.C.*	MG. PER 100 C.C.	MG. PER 100 C.C.	
1933 Nov. 16	20.9 39.4 23.4	23.4	100	
Dec. 28			600	
1934 Jan. 10	18.0 42.8 26.0	26.3	400	2 Granular casts with fat, one with cells. 6 Cylindroids
Feb. 8			700	
Feb. 9	20.9 34.5 23.5	28.3	700	2 Granular casts
Mar. 6	22.9 34.8 26.6	29.4	800	1 Granular cast
April 10	32.2 42.8 32.0	29.1	800	47 Granular casts, all with fat, 10 with cells. 40 Cylindroids
May 8	21.6 45.7 37.4	36.0	700	1 Granular cast with fatty cells
May 9		40.0		

*Initial, one-and-one-half-hour and five-hour values.

History of this rat: Born Sept. 4, 1933. On stock diet Oct. 17, liver diet Oct. 31. Left kidney ligated Oct. 17. Right nephrectomy Oct. 31. Killed May 9, 1934. Body weight 395 gm. Kidney 23 x 18 x 12 mm. Weight 2.15 gm. One-third of kidney absorbed. Marked atrophy beyond ligature. Slight early nephritis.

Protocol II gives data on a representative rat whose kidney showed no histologic evidence of nephritis. Here again there are apparently normal xylose curves, although albumin goes up from 20 to 250 mg. per 100 c.c. and granular casts and cylindroids are reported. The erratic nature of the xylose curves is again shown. Urea values show no progressive degree of retention such as is shown in Protocol I.

The pathologic report on the rat livers is interesting. The large majority have what is commonly called "albuminous degeneration," i.e., the liver cells are full of pinkish staining granules, and they appear swollen. This may be

PROTOCOL II

EXPERIMENTAL FINDINGS WITH RAT 791 ♀. NO EVIDENCE OF NEPHRITIS

DATE	BLOOD		URINE	
	XYLOSE NFM	UREA N	ALBUMIN	SEDIMENT
	MG. PER 100 C.C.*	MG. PER 100 C.C.	MG. PER 100 C.C.	
1933 Nov. 22			20	
1934 Jan. 5	21.7 29.8 23.8	27.5	250	
Feb. 8	18.6 39.8 19.9	21.3	200	2 Granular casts with fat and cells 9 Cylindroids
Mar. 7	21.3 30.2 27.1	25.8	400	2 Cylindroids
April 11	23.7 38.4 23.3	27.0	600	1 Granular cast with fat and cells 9 Cylindroids
May 8	19.3 39.0 32.6	23.9	2000	1 Granular cast with fat
May 9		23.8		

*Initial, one-and-one-half-hour and five-hour values.

History of this rat: Born Sept. 8, 1933. On stock diet Oct. 23, liver diet Nov. 2. Left kidney ligated Oct. 23. Right nephrectomy Nov. 2. Killed May 9, 1934. Body weight 320 gm. Kidney 24 x 13 x 12 mm. Weight 1.65 gm. One-half of kidney absorbed. Slight atrophy beyond ligature. No evidence of nephritis.

due to some toxic effect of the xylose on the liver cells. In this connection, it may be well to report that the rats appeared quite ill after the ingestion of xylose, and diarrhea was common. The xylose test was given to various members of the laboratory staff and the majority reported severe diarrhea.

Fishberg and Friedfeld, working with patients manifesting kidney lesions and rabbits with uranium-induced nephritis, report that after the ingestion of xylose, the blood curve of nonfermentable reducing substances, instead of approaching the normal fasting value after five hours, continues upward. The writer has performed some 40 xylose clearance tests, using rabbits with uranium-induced nephritis. In none of these did the nonfermentable sugar of the blood continue upward after the second hour, and practically all fifth-hour values approached the fasting normal. In seven instances the fifth-hour value was down about 50 per cent from the maximum. In many cases the normal clearance of the animal checked fairly well with the clearances after the animal had repeatedly been injected with uranium. Eighteen animals were used in the experiment. Some received as many as six injections of uranium in increasing dosage up to 1 mg. per kilo body weight. Histologic examination showed severe kidney lesions.

SUMMARY

The results of these experiments are consistent with the view generally held that kidney function must be seriously disturbed before there is retention of urea. The degree of nephritis produced was not enough to cause marked histologic changes. The values for blood xylose indicate that the use of this sugar as a delicate index of renal function is open to serious question. A more severe

nephritis than that reported here must be produced before consistent decreases in the excretion of xylose can be expected. The excretion of albumin and the presence of casts in the urine still appear to be the earliest indicators of renal disturbance.

The author is indebted to Dr. E. M. Medlar for the pathologic work. He wishes to express his appreciation to Dr. N. R. Blatherwick for helpful criticism, and to Dr. J. M. Connolly and Phoebe Bradshaw for valuable assistance in the work. Pure xylose was generously furnished by the United States Bureau of Standards.

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COMPARATIVE STUDIES IN CHEMOTAXIS*

JACOB E. KLEIN, M.D., CHICAGO, ILL.

CHEMOTAXIS is a biologic phenomenon of great significance in immunity and inflammation. There are still controversial questions in the study of this reaction. It is known that various chemical substances may exert a chemotactic influence on leucocytes. Wells[1] mentions the significance of the H-ion concentration and states that leucocytes tend to migrate toward the point of greater H-ion concentration. Heinsheimer,[2] who studied the effect of acidosis and alkalosis on the blood leucocytes, thought that acidosis increased the cells of the myeloid series, while alkalosis increased the cells of the lymphatic series, and ascribed the cause of this response to probable variations in stimulation of the sympathetic and parasympathetic nervous system. In his experiments Heinsheimer induced acidosis by inhalation of CO_2, and alkalosis by injection of sodium carbonate until the blood pH rose from 7.32 to 8.52. Gabritschewsky[3] (quoted by Wells) studied the chemical influence of a large number of substances on leucocytes and classed them in three groups: (1) Substances exerting "negative chemotaxis" including those that attracted only a few leucocytes, such as concentrated solutions of sodium and potassium salts, lactic acid in all concentrations; (2) substances with "indifferent chemotaxis," which attracted moderate numbers of leucocytes, such as distilled water, dilute solutions of sodium and potassium salts, peptone, glycogen, and bile; (3) substances with marked positive chemotaxis, such as papayotin, and sterilized, as well as living cultures of bacteria, whether pathogenic or nonpathogenic. The substances of this group produced general as well as local leucocytosis when injected subcutaneously.

A review of the literature indicates that no law has been determined which accounts for the presence or absence of chemotactic influence in a given substance, since closely related chemical substances may induce entirely opposed chemotactic reactioins, while dissimilar chemical substances may display similar properties as concerns chemotaxis.

In the following studies an attempt has been made to compare the local histologic reaction and the general response of the blood, particularly the leucocytes, after the subcutaneous injection of a dilute inorganic acid, a dilute alkali, a dilute organic acid, a typical hormone, and a characteristic bacterial toxin.

EXPERIMENTS

Eight guinea pigs, average weight 300 gm., were prepared by carefully shaving the skin over the lateral part of the abdomen, using the same relative region in each animal. After the area had been carefully washed, and swabbed with mercurochrome solution, the substance to be tested was injected subcutaneously.

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At the same time total leucocyte and differential counts were done on each animal. When the experiment was terminated each animal was chloroformed and the tissue carefully excised, fixed in 10 per cent formalin, mounted in paraffin and stained with hematoxylin—eosin, as well as with Wright's stain for special cytologic study. In this way the local tissue reaction was compared with the general reaction as shown by the blood studies. The substances used and compared were dilute HCl (1-1,000), dilute NaOH (1-1,000), lactic acid, 2 per cent hog thyroglobulin, and 1/5 M.L.D. of diphtheria toxin. As indicated in Figs. 1 to 4, guinea pig No. 1 received 1 c.c. of dilute HCl subcutaneously and was killed after twenty-four hours; No. 2 was given 1 c.c. of dilute NaOH and was killed after twenty-four hours; No. 3 received a daily injection of 1 c.c. of dilute HCl for a week; No. 4 received the same amount of dilute NaOH daily

Fig. 1.

Fig. 2.

Fig. 1.—Dilute HCl, twenty-four-hour reaction. Lymphocytes and polymorphonuclear cells.

Fig. 2.—Normal skin and subcutaneous tissue after injection of 1-1,000 NaOH.

for one week; No. 5, 1 c.c. of 1-1,000 lactic acid daily for one week; No. 6 was given ½ c.c. of 2 per cent hog thyroglobulin daily for one week; No. 7 received 1 c.c. of diphtheria toxin (⅕ M.L.D.) daily for one week.

DESCRIPTION OF LOCAL REACTION

Microscopic study of the tissues (see drawings) revealed the following changes:

1. Dilute HCl, twenty-four-hour reaction (Fig. 1). A considerable infiltration with lymphocytes was present and a moderate polymorphonuclear reaction. A count of 100 cells in a Wright stained specimen showed 82 per cent lymphocytes and 18 per cent polymorphonuclear cells.

2. 1-1,000 NaOH for twenty-four hours showed no local reaction whatever (Fig. 2). The normal connective tissue reticulum of the subcutaneous tissues was undisturbed.

3. 1-1,000 HCl daily injections for one week. There was a dense leucocytic infiltration in the subcutaneous tissues with a predominance of lymphocytes and reticulum cells. It was a "sterile" abscess.

4. 1-1,000 NaOH daily injections for one week. Normal skin and subcutaneous tissue. No inflammatory reaction whatsoever.

5. 1-1,000 lactic acid daily injections for one week. There was marked lymphocytic infiltration in the subcutaneous tissue. Lymphocytes predominated. No unusual cells were seen.

6. One-half cubic centimeter of 2 per cent hog thyroglobulin daily for one week. There was a marked infiltration of the subcutaneous tissues. Intense hyperemia was due to the marked dilatation of the subcutaneous capillaries. There was observed extreme predominance of lymphocytes in the tissues. A differential count on a Wright stain of the tissues indicated 100 per cent lymphocytes. In the areas with dilated capillaries, they could be seen in various stages passing through the capillary wall (Fig. 3).

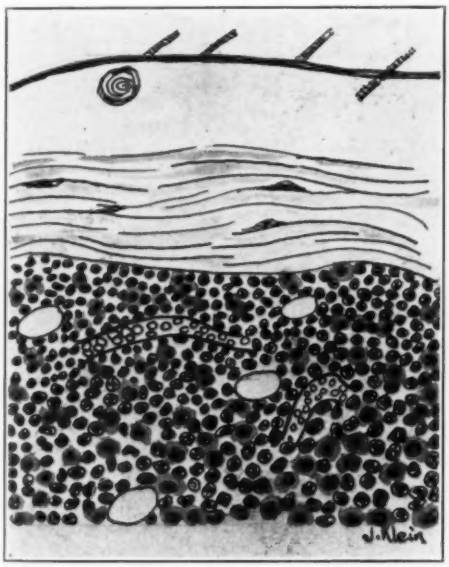

Fig. 3.

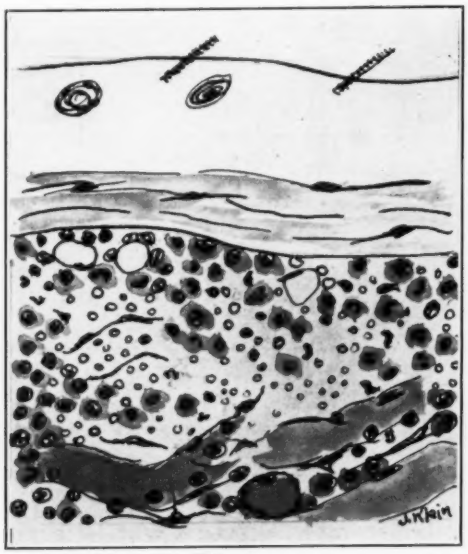

Fig. 4.

Fig. 3.—Lymphocytic infiltration after injection of thyroglobulin.

Fig. 4.—Injection of diphtheria toxin. Local necrosis, hemorrhage and inflammatory reaction.

7. Received 1 c.c. (1/5 M.L.D.) of diphtheria toxin daily for one week. The subcutaneous tissues showed extreme infiltration with lymphocytes and polymorphonuclear cells. Many leucocytes were seen with ingested erythrocytes. Necrosis of subcutaneous tissue and muscle was marked. Extravasation was extreme throughout the subcutaneous tissue. A differential count on these cells gave 26 per cent lymphocytes. No eosinophiles or other unusual cells were noted.

8. A normal guinea pig skin used for control showed no unusual changes.

THE GENERAL REACTION

The general reaction as indicated by the leucocyte and differential count corresponded very much with the local tissue reaction (see charts of blood counts, Table I).

1. 1-1,000 HCl, twenty-four hours, mild leucocytosis.

2. 1-1,000 NaOH, twenty-four hours, no change.

3. 1-1,000 HCl, week, moderate leucocytosis.

4. 1-1,000 NaOH, week, no change.

5. 1-1,000 lactic acid daily for one week. There was practically no noteworthy change in the total leucocyte count, which might be ascribed to normal variations. The differential count indicated a moderate increase in the polymorphonuclear cells.

6. One-half cubic centimeter of 2 per cent hog thyroglobulin for one week. The blood count showed considerable leucocytosis with a predominance of the lymphocytes.

7. One-fifth M.L.D. of diphtheria toxin daily for one week showed a marked increase in the white count with an increase in the percentage of polymorphonuclear cells.

TABLE I

GUINEA PIG	PRELIMINARY COUNT						24 HOURS AFTER INJECTION					
NO. 1	W.B.C.	P.	S.M.	L.M.	BAS.	TRANS.		P.	S.M.	L.M.	BAS.	TRANS.
1-1,000 HCl 24 hours' duration	6,000	5	94	0	0	1	10,400	10	89	0	1	0
NO. 2	W. B. C.											
1-1,000 NaOH 24 hours' duration	10,400	4	93	0	0	3	10,600	45	52	0	0	3
NO. 3	DAILY BLOOD COUNTS						1 HOUR AFTER INJECTION					
1-1,000 HCl		P.	S.M.	L.M.	BAS.	TRANS.		P.	S.M.	L.M.	BAS.	TRANS.
1 week's duration	5,800	28	68	3	0	1	3,000	30	63	3	0	4
	7,800	11	85	3	0	1	4,200	10	86	3	0	1
	7,000	13	82	4	0	1	3,400	8	90	2	0	0
	5,400	9	90	1	0	0	5,400	24	70	3	0	3
	9,000	23	75	0	0	2	7,000	35	65	0	0	0
	10,600	18	81	1	0	0	13,600	12	85	13	0	0
	15,600	16	83	1	0	0	14,600	12	88	0	0	0
NO. 4	7,800	8	92	0	0	0	5,000	14	85	1	0	0
1-1,000 NaOH	9,600	5	93	2	0	0	5,200	9	87	2	0	2
1 week's duration	11,200	26	73	1	0	0	5,000	16	83	0	0	1
	7,000	12	88	0	0	0	8,600	9	91	0	0	0
	8,600	10	90	0	0	0	9,800	25	65	0	0	0
	8,000	11	89	0	0	0	7,400	20	80	0	0	0
	9,600	20	80	0	0	0	8,400	22	78	0	0	0
NO. 5	9,600	8	92	0	0	0	7,600	49	51	0	0	0
1-1,000	9,400	4	96	0	0	0	10,800	16	82	2	0	0
lactic acid	8,200	16	83	0	0	1	8,800	41	59	0	0	0
1 week's duration	9,600	60	40	0	0	0	12,800	14	85	0	0	0
	9,200	17	8	0	0	2	10,600	44	55	0	0	0
	8,600	13	84	0	0	3	8,400	16	80	1	0	1
	8,200	72	23	0	0	1	9,800	50	47	1	0	2
NO. 6	7,600	6	92	0	0	2	4,600	10	88	0	0	2
Thyroglobulin	6,600	18	79	0	0	2	6,000	4	94	0	0	2
½ c.c.	9,000	22	78	0	0	0	8,000	2	98	0	0	0
2 per cent	11,800	18	82	0	0	0	10,400	11	87	1	0	1
daily	9,600	17	82	0	0	1	7,600	13	87	0	0	0
	9,800	20	80	0	0	0	10,800	15	83	0	0	2
	15,200	24	76	0	0	0	14,600	40	60	0	0	0
NO. 7	15,800	18	82	0	0	0	19,000	24	75	1	0	0
1 c.c.	18,000	14	86	0	0	0	15,400	54	46	0	0	0
Diphth. toxin	22,600	9	91	0	0	0	23,200	48	52	0	0	0
1 week's duration	24,200	33	66	0	1	0	24,800	22	77	0	1	0
	10,400	15	84	0	0	1	19,600	23	76	1	0	0
	19,400	34	66	0	0	0	18,200	40	60	0	0	0
	11,000	9	91	0	0	0	13,000	34	63	0	2	1

DISCUSSION OF RESULTS

Dilute alkali induced no change in the local or general reaction. As might be expected dilute HCl was quite irritating and provoked both a local and general reaction. Lactic acid had a greater local effect than the blood count would seem to indicate. It was interesting to find that thyroglobulin provoked a violent lymphocytic reaction and dilatation of the tissue capillaries, almost equal in extent with that induced by $\frac{1}{5}$ M.L.D. of diphtheria toxin, although not associated with hemorrhage and necrosis, as was the latter. The most severe local and general reaction was caused by diphtheria toxin which raised the leucocyte count to 24,000 and increased the proportion of polymorphonuclear cells. Thyroglobulin induced a fair increase in leucocytes (7,600 to 15,000) and a relative lymphocytosis. The latter finding may explain the tendency to lymphoid hyperplasia in exophthalmic goiter, where even the thyroid tissues are infiltrated with lymphocytes. It seems that a substance which causes a local inflammatory reaction, usually induces a general response as shown by the blood count, although there may be exceptions as is the case with lactic acid. The most common cell reaction was lymphocytic in nature. Hydrochloric acid and diphtheria toxin affected the polymorphonuclears also. No unusual cells, such as eosinophiles or giant cells, were seen. Of practical interest is the lack of reaction to alkali. When hypodermic and subcutaneous medication is given it would seem advisable to have an alkaline reaction, if possible.

SUMMARY

A comparison was made of the local and general effects of the subcutaneous injections of 1-1,000 NaOH, 1-1,000 HCl lactic acid, thyroglobulin, and diphtheria toxin. The alkali produced the least and diphtheria toxin the most extensive reaction. Of particular interest was the marked lymphocytic local and general response induced by thyroglobulin.

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3917 Gladys Avenue

CONSTITUTION AND ARTHRITIS*

JOSEPH KOVACS, M.D., AND EDWARD F. HARTUNG, M.D., NEW YORK, N. Y.
WITH THE TECHNICAL ASSISTANCE OF VIRGINIA HANSCOM, A.B.

THE modern American viewpoint in arthritis (as in most disease states) is topical when approaching it from the aspects of research, diagnosis, or treatment. This has definite but only partial value, as in the case of focal infection and vaccine therapy in relation to arthritis. No matter from what angle we approach the study of disease, an understanding of constitution and its relation to pathologic processes is important. However, the term constitution connotes very little that is tangible to our minds, involving as it does those imponderable quantities we call heredity, immunity, and the vagaries of the endocrine and vegetative nervous systems.

There are two factors at work in the formation of an individual's constitution. One of these, comprising the qualities inherent in the germ plasm, is called the genotypic factor. The other, the paratypic factor, expresses the effect of adverse or useful environmental forces and modifies the character of the organism from the first moment of life. These two sets of agencies are continually modeling the individual, and the final result is the patient as he presents himself to us.

In Europe, especially on the continent, great emphasis has been placed on constitution as the ultimate determining factor in disease, and its resultant pathologic picture. The viewpoint is frequently held that the same etiologic factors playing in an identical manner on varying constitutional make-ups, will produce different types of diseases, different clinically and pathologically. Here in America, while the idea is by no means foreign to us, it is too often neglected. If it is true that certain constitutions predispose to certain diseases (and this is commonly held in such conditions as tuberculosis, peptic ulcer, and gall-bladder disease), then a more complete knowledge of these various constitutions is of extreme importance in connection with such a disease as chronic arthritis.

The earlier workers on arthritis, notably Heberden (1802), Haygarth (1805), and Robert Adams (1857), neglected to observe, or at least failed to note, any association of constitutional states with the disease. The first notable mention of the subject was by Bryant and Goldthwaite[1] (1909), who pointed out the association of the light-boned, slender, asthenic, visceroptotic type with rheumatoid arthritis. As a matter of fact, such an observation only became possible after the sharp differentiation pathologically in the early part of the century between rheumatoid arthritis and osteoarthritis. Since that time, the fact has been more frequently pointed out that rheumatoid arthritis occurs predominantly in the asthenic type of constitution and osteoarthritis in the pyknic

*From the Arthritis Clinic of the Department of Medicine of the New York Post-Graduate Medical School and Hospital.
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type (Osgood,[2] Swaim[3]). The etiology of these two diseases is still a matter of serious dispute. Are they, as claimed by most, two different diseases, or, as claimed by a few, the same disease manifesting itself differently in different constitutional types?

A study of constitution in arthritis will be of value, for although it will not reveal any new facts not already described in general terms by observing physicians, nevertheless, it will present these facts on a mathematical basis. There are many approaches to constitution, the most obvious ones being morphologic, physiologic, immunologic, and psychologic. We have chosen the morphologic and psychologic methods of approach. On the same patients on which this study is based, other factors of constitution are in the process of investigation and will be the subject of subsequent papers.

The modern constitutional study is based on the acknowledgment of two types, known from ancient times as habitus apoplecticus and habitus phthysicus. They represent the extreme antithetic variation from the median type. We find them in every race and at every age, in women as well as in men. Function, morphology, and the predisposition to disease are antagonistic in the two antithetic body types. Contrary to the older intuitive method of recognizing the two constitutional types, the anthropometric method, inaugurated by Johannis Sigismundi Elsholzius,[4] in the year 1654 and perfected by Achille di Giovanni[5] in 1800, is based on numerical determinations. Since then, Viola[6] and Nicola Pende[7] in Italy, Martius,[8] Krause,[9] and Bauer,[10] in Germany, Bryant,[11] Bean,[12] and Draper[13] in America, and others, have perfected the technic. The nomenclature in constitutional studies is confusing, as nearly every worker has his own. We have used that of Borchardt,[14] which is more generally accepted and which differentiates between asthenic (the old habitus phthysicus) and pyknic (the old habitus apoplecticus) type of individuals.

Di Giovanni was the first who attempted to describe the morphologic configuration of those who suffered with arthritis. Lack of a clear-cut conception was due to the poor differentiation of the manifold rheumatic manifestations as we know them today, especially the distinction between rheumatoid arthritis and osteoarthritis—the two rheumatic conditions in which constitutional differentiation is most clear-cut.

METHOD

We took in sequence of their arrival at the clinic, 50 cases of osteoarthritis and 50 cases of rheumatoid arthritis and subjected them to careful anthropometric and psychologic study. All were females. The criteria on which one bases the differentiation between rheumatoid arthritis and osteoarthritis are now fairly well understood, the main difficulty being those cases which are designated "mixed," where osteoarthritis and rheumatoid arthritis are seen at the same time in the same patient. In this study we tried to include only those cases purely of one or the other type.

The technic of measurement was based largely on that of Martin,[15] as outlined in his *Textbook of Anthropology*, but suggestions were drawn also from the works of Draper.[13] We made a few relatively unimportant modifications. Having a general idea of the different constitutional types found in chronic

arthritis, we decided on a reduction in the number of measurements, and our experience has led us to believe that we have taken all the measurements which seem to be of importance in the differentiation of constitutional types in rheumatoid arthritis and osteoarthritis. In addition, we added the span-height, upper extremity-height, lower extremity-height, chest circumference-height, abdomen circumference-height and neck length-height indices to the generally accepted neck length-neck circumference, thoracic anteroposterior diameter-thoracic lateral diameter, and cephalic length-cephalic breadth indices.

We have not compared our findings with the so-called normal type. It is very difficult in a hybrid race, such as we are dealing with in America, to establish an acceptable standard. The literature gives no help in determining normal figures. If we compare Viola's[6] so-called normal or median measurements taken on the Venetian female, or Rott's[16] median measurements taken on the German female, with Draper's[17] measurements taken on the average American female, we get decidedly different results. For these reasons, we have used the method suggested by Draper, comparing various disease groups, and have tried to define the various characteristic differences.

For better comparative study, we also divided the patients into age groups of ten years, and compared the constitutional findings in these groups. Unfortunately, we had only sufficient material for comparison between the age groups of forty-one to fifty and fifty-one to sixty. The ages of the examined 50 osteo- and 50 rheumatoid arthritic individuals were as shown in Table I.

TABLE I

	AGE						TOTAL
	11-20	21-30	31-40	41-50	51-60	61-70	
Rheumatoid arthritis	2	6	16	16	10	–	50
Osteoarthritis	–	–	4	15	18	13	50

The anthropometric measurements gave the results (in centimeters) shown in Tables II and III.

Comment.—An analysis of the results of our study demonstrated that the weight of the rheumatoid arthritic is, on an average, twenty pounds less than that of the osteoarthritic individual.

Another interesting finding is that the osteoarthritic group has a longer span length than the rheumatoid arthritic group, and in many cases the span length exceeds the standing height, showing a tendency to increased horizontal measurement. We did not find marked differences between the standing height, and upper and lower extremity length in the two groups of arthritic individuals. Furthermore, we did not find pronounced difference between the head measurements, except that the osteoarthritic has a somewhat longer and slightly narrower head. On the other hand, there are marked differences in the neck length and neck circumference in the two types of arthritis. The osteoarthritic person has a large and short neck; the rheumatoid has a long and small neck. The neck of the rheumatoid is, on the average, 1.2 cm. longer and its circumference is, on an average, 2.2 cm. less than that of the osteoarthritic person. We found also a marked increase in the measurements of the thoracic anteroposterior diameter, thoracic lateral diameter, chest circumference and abdomen circumference in the

osteoarthritic group; the thoracic diameter is around 1 or 2 cm. greater, the chest circumference around 9 or 10 cm. larger, and the abdomen circumference around 4 cm. larger. Also, the bi-iliac diameter is around 2 cm. wider in the osteoarthritic group. We did not find pronounced differences in the umbilicus-pubis and xiphoid-umbilicus lengths of the two groups. The average showed some

TABLE II

ANTHROPOMETRIC MEASUREMENTS OF 50 RHEUMATOID AND 50 OSTEOARTHRITIC INDIVIDUALS

	RHEUM. ARTHR.	OSTEO-ARTHR.		RHEUM. ARTHR.	OSTEO-ARTHR.
Age	40.84	54.02	Abdomen circumference	86.26	90.62
Weight	138.87	158.24	Bi-iliac diameter	26.44	28.70
Span	154.34	156.38	Umbilicus-pubis length	15.09	15.86
Standing height	156.61	156.47	Xiphoid-umbilicus length	16.80	18.51
Upper extremity length	68.71	69.39	Cephalic length-breadth index	86.05	83.02
Lower extremity length	89.19	89.70	Neck length-neck circumf. index	39.60	33.20
Cephalic length	17.84	18.16	Neck length-stand. height index	7.90	7.00
Cephalic breadth	15.31	15.16	Upper extrem.-stand. height index	43.87	44.34
Head circumference	53.76	54.84	Lower extrem.-stand. height index	56.90	57.30
Neck length	12.44	11.20	Thoracic index	75.90	75.60
Neck circumference	31.40	33.67	Span-stand. height index	98.50	99.90
Thoracic ant.-post. diameter	18.26	20.79	Abdomen circumf.-stand. height index	55.00	57.90
Thoracic lateral diameter	25.37	26.78	Chest circumf.-stand. height index	54.00	60.00
Chest circumference	84.59	94.01			

TABLE III

ANTHROPOMETRIC MEASUREMENTS OF THE AGE GROUPS OF FORTY-ONE TO FIFTY AND FIFTY-ONE TO SIXTY

	AGE FORTY-ONE TO FIFTY		AGE FIFTY-ONE TO SIXTY	
	RHEUM. ARTHR.	OSTEO-ARTHR.	RHEUM. ARTHR.	OSTEO-ARTHR.
Weight	134.53	161.62	126.19	156.82
Span	154.80	159.50	151.77	155.22
Standing height	157.07	158.86	155.72	155.27
Upper extremity length	69.22	71.34	69.05	68.66
Lower extremity length	90.05	89.80	88.42	90.35
Cephalic length	17.87	18.19	17.86	17.89
Cephalic breadth	14.84	15.13	15.20	15.09
Head circumference	54.17	54.98	54.37	54.34
Neck length	12.21	10.65	11.78	11.14
Neck circumference	31.62	33.38	31.44	33.27
Thoracic ant.-post. diameter	18.42	20.71	17.92	20.72
Thoracic lateral diameter	26.34	27.93	25.19	26.72
Chest circumference	84.98	96.00	86.03	92.47
Abdomen circumference	89.72	100.47	88.30	99.32
Bi-iliac diameter	26.94	29.17	27.84	28.66
Umbilicus-pubis length	14.76	16.15	14.86	15.72
Xiphoid-umbilicus length	17.18	18.48	17.24	19.21
Cephalic length-breadth index	83.60	83.17	85.16	84.30
Neck length-neck circumference index	38.62	31.90	37.43	33.42
Neck length-stand. height index	7.82	6.73	7.64	7.10
Upper extrem.-stand. height index	44.03	44.92	44.30	44.23
Lower extrem.-stand. height index	57.60	56.52	56.74	57.60
Thoracic index	69.92	75.58	71.14	77.56
Span-stand. height index	98.52	100.03	97.43	99.96
Abdomen circumf.-stand. height index	57.12	63.28	56.73	63.92
Chest circumf.-stand. height index	54.13	60.45	55.23	59.50

differences, but both measurements varied so greatly in the individuals that we did not find any characteristic property which would help in the differentiation of the two groups.

Characteristic of the rheumatoid group is the comparatively greater neck length-neck circumference index. The neck length-height index does not give so marked a difference; still, this index is increased, demonstrating that neck length compared to the body length is longer in the rheumatoid group.

The upper and lower extremity-height indices, and the thoracic anterior posterior-lateral diameter indices did not show marked differences. On the other hand, the span-height index is increased and close to one in the osteoarthritic group.

The abdomen circumference-height and chest circumference-height indices are decidedly increased in the osteoarthritic group.

When we compare our anthropometric findings by age groups of forty-one to fifty and fifty-one to sixty, we get practically the same results. In the age group of forty-one to fifty the weight difference is around 27 pounds, and in the age group of fifty-one to sixty, over 30 pounds. These findings show that in the osteoarthritic group there is a tendency to fat deposit with the advance of age.

We find a decided difference between the two types of arthritis if we use the Pignet index ($X = H - (C + W)$ as a comparative measure, H representing standing height, C chest circumference, and W body weight, all in metric units.

PHYSICAL DEVELOPMENT	OSTEO-ARTHRITIS	RHEUMATOID ARTHRITIS
6 +	84% } 90%	18% } 30%
5 +	6% }	12% }
4 +	0	20%
3 +	8%	16%
2 +	2%	16%
1 +	0	18%

Thus we see that in osteoarthritis 90 per cent have well-developed constitutions, according to the Pignet index, compared with only 30 per cent in the rheumatoid group.

PSYCHOLOGIC ANALYSIS

An attempt was made to determine differences psychologically between the rheumatoid and osteoarthritis groups. Psychologic classification of people falling within the normal range of variation is extremely difficult, and made still more difficult by the transient contact of the investigator with ambulatory patients. We used a method of analysis devised by Dr. Smiley Blanton. The 100 cases mentioned above were classified according to this outline. A conscientious effort was made to make adequate mental contact with each patient.

An analysis of our tables shows that we were unable to demonstrate any marked difference psychologically between these two groups. Whether this is a fact or whether our analytical approach was faulty, we are unable to say. Since the tables are large and the results negative, we did not think it worth while to publish them here.

CONCLUSIONS

Anthropometric studies of 50 rheumatoid arthritic and 50 osteoarthritic patients reveal marked differences in constitutional morphology. The most outstanding are that:

The rheumatoid group has a tendency to increased longitudinal measurements. Individuals of this group have decidedly longer and thinner necks, they are mostly slender, though not necessarily tall; their weight is around normal or under the average.

The osteoarthritic group has a tendency to increased horizontal measurements. Individuals of this group have shorter, thick necks, their silhouette is more massive than slender, their chest circumference in relation to abdomen circumference is comparatively greater, the thoracic anteroposterior diameter being by far the larger. They have a tendency to be overweight.

No psychologic differences were found between the rheumatoid and osteoarthritic groups.

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THE EFFECTS OF DYES ON ENDAMEBA HISTOLYTICA IN VITRO*

H. TSUCHIYA, SC.D., ST. LOUIS, MO.

THOUGH the effects of dyes on free-living protozoa were studied by various workers, Koch[1] was probably the first to systematically investigate the problem on parasitic protozoa. She undertook to determine dilutions of various dyes lethal to trophozoites of *Endameba gingivalis* and concluded that the action of dyes on the organisms in vitro was amebicidal rather than amebastatic. According to her investigations the phenol-methanes were found to be the least amebicidal, while the diphenyl-methanes were highly lethal to the amebas. Howitt[2] previously observed that acriflavine was apparently as toxic for *E. gingivalis* in vitro as certain arsenical compounds, but the inhibitory effect on the growth was lost when the dye was diluted beyond 1:142,000.

Tripoli[3] introduced cysts of *E. histolytica* into culture medium containing 0.1 c.c. of 1 per cent aqueous solution of acriflavine and claimed to have succeeded in growing amebas by reducing bacterial population in the culture. Browne[4] recently demonstrated that 1 per cent aqueous solutions of neutral red and janus green were toxic to *E. histolytica*, while trypan blue and Nile blue were not in concentrations lower than 0.5 per cent.

Since bacteria-free cultures of amebas are not available at present, the effects of dyes in reality involve both the accompanying bacteria as well as amebas present in culture. Variations in the composition and reaction of the medium as well as the accompanying bacterial flora may, therefore, be taken as factors responsible for differences in results of various investigators. Koch claimed that trophozoites of *E. gingivalis* were able to live in cultures with a minimum bacterial content, but the nature of relationship between the bacteria and protozoa was not discussed by her. That such relationship might be of importance was well brought out by Cleveland and Saunders[5] in their study on *E. histolytica*. These authors believe that the amebas were able to multiply very rapidly in vitro when *Escherichia communior*, *Vibrio comma*, and *Neisseria catarrhalis* were present.

The present study was primarily undertaken to determine the viability of cysts and trophozoites of *E. histolytica* in vitro when exposed to selective action of various dyes; second, it was hoped that it may be possible to ascertain whether the presence of dyes may cause biologic variations of amebas, particularly the development of "dye-resistance."

MATERIALS AND METHODS

Washed Cysts.—The strain of cysts of *E. histolytica* studied came from a carrier on whom observations on "encystment cycle" were previously made.[6]

*From the Department of Bacteriology, Immunology and Public Health, Washington University School of Medicine.

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Washed cysts were prepared according to the method described by me[7] and were found to contain considerably fewer bacteria than were present in the original stool.

Viability Test.—The use of 0.1 per cent aqueous solution of eosin to determine the viability of the cysts was found to be quite inadequate in this study. In one instance 96 per cent of washed cysts stored at 5° C. as long as 115 days were found to be unstainable, though no growth in culture mediums could be secured from this material upon repeated attempts. This seemed to indicate that, while all of the stained cysts were dead, those unstained were not necessarily viable. Throughout this study, therefore, the viability of the cysts was ascertained by subculturing on the S. C. medium[7] with Dorsett's egg slant. The amebas in this medium usually attained an optimum growth after an incubation period of eighteen to twenty-four hours at 37° C.

Dyes and Technic.—The following dyes were used in this study: (1) Triphenyl-methane dyes: gentian violet, malachite green, brilliant green, pyoktanin, acid and basic fuchsins. (2) Acridine dye: acriflavine. (3) Quinoneimine dyes: neutral red, methylene blue, safranin, and brilliant cresol blue and toluidine blue. (4) Azo dye: Bismarck brown. (5) Xanthene dyes: eosin B and mercurochrome (a combination of fluorescein and mercury).

To 5 c.c. of each dye dilution was added 0.1 c.c. of washed cysts, and the mixture was kept at varying temperatures (5° C., 22° C., and 37° C., respectively). At varying time intervals following exposure to the dyes, 0.1 c.c. of each mixture was transferred aseptically, after thorough shaking, into a tube containing 0.9 c.c. of sterile nutrient broth to dilute the dye and this prevents its further action on the amebas. One-tenth cubic centimeter of the resulting broth dilution was introduced into the culture medium and incubated at 37° C. From time to time a few drops of the sediment were withdrawn from the bottom of the culture medium by means of a pipette (graduated to tip). A single drop, which approximated 0.2 c.c. of the material, was then examined immediately under the microscope with respect to the extent of growth and motility of the organisms. The degree of dye permeability in the amebas was noted in each case with the view to corroborating the permeability of the dye with the viability of the cysts in culture.

Permanent preparations of the amebas were made either by Heidenhain's iron hematoxylin method or by the method recently described by me.[8] Morphologic variations of the organisms, if any, were carefully looked for, and recorded.

The Gram stained smears of culture materials were then studied with a view to ascertain the relative predominance of gram-positive or gram-negative bacteria. The relative proportion of unchanged starch granules in culture medium after incubation was considered as indicative of the extent of growth of the starch-splitting types of organisms.

To study the effect of dyes upon the trophozoites of *E. histolytica*, 0.5 c.c. of each dilution of dye was added to 4.5 c.c. (liquid portion) of a culture medium which maintained luxurious growth of the amebas, thus making the final dilution one-tenth of the original. Observations were then carried out at 24, 48, and 72 hours' incubation at 37° C. Furthermore, the amebas that survived in primary culture were transferred to fresh culture medium, and then

exposed to a similar concentration of the dye as those used previously. This process was repeated from day to day in order to determine if the so-called dye-resistant strain would subsequently develop during the course of successive transfers of the organisms.

OBSERVATIONS

The results of the exposure of cysts to different concentrations of the dyes showed that after exposure to certain concentrations of the dyes, the cysts which remained viable were determined by subculturing on the culture medium. However, the growth of the organisms in the media appeared after more or less prolonged period of lag as recorded in Table I. It was observed that in these mixtures in which the lag period was the longest (amebastasis), dyes permeated into a certain proportion of the cysts. In the mixture in which the cysts were exposed to high concentration of the dyes, the viability of the cysts was lost as determined by the absence of the growth of the organisms in cultures, and the dyes permeated all of the cysts.

Amebastatic effect was best shown by gentian violet, acriflavine and malachite green (lag periods of 72 and 68 hours), and basic and acid fuchsins to a lesser extent. On the other hand, amebas grew abundantly in culture medium after the exposure to 1:100 dilution of safranin. As a matter of fact, the growth even surpassed that seen in the control tube, while in the case of neutral red, there was neither inhibition nor stimulation of the growth of the organism. The remainder of the dyes exerted amebastatic effect to an intermediate degree. Thus, following the exposure to 1:400 dilution of eosin B, 1:800 dilution of Bismarck brown, 1:1,600 dilution of brilliant green and 1:6,400 of mercurochrome, the growth of the amebas was initiated after lag period of forty-eight hours. In the case of toluidine blue, brilliant cresol blue and pyoktanin, the incubation period was found to be thirty-six hours.

Gentian violet exhibited the amebastatic effect on the cysts of *E. histolytica*. Thus, by the introduction of 1:200 dilution of the dye, the initial growth of the organisms was not observed until an incubation period of seventy-two hours was reached. The amebas in the culture medium apparently were able to maintain a good growth for at least six days without further transfer. The individual organism showed a considerable increase in size both of the cytoplasm and to a slighter extent of the nucleus. Differences in size were so significant that the identity of the organisms might be questioned by casual observers. This might be explained on the ground that the selective action of the dye was such that incidental bacteria were affected without injury to the amebic protoplasm. This action, further, resulted in the prolongation of the life of the culture, so that without transferring or changing the medium in any way, the organisms remained actively motile and undergoing division for several days (see Table I). During this period of active growth of the amebas, surviving bacteria increased in number, and the amebas gradually returned to their normal size. Transfer of this culture to fresh medium resulted in a normal growth of the organisms.

In the cultures exposed to malachite green gram-positive bacteria were inhibited, whereas in those exposed to acriflavine the growth of gram-negative bacteria was inhibited. It was noted in general that starch granules remained undigested in this medium which indicated that the starch-splitting types of

TABLE I

EFFECTS OF DYES ON CYSTS OF ENDAMEBA HISTOLYTICA IN VITRO

DYES		AMEBICIDAL ZONE (IN DILUTIONS)	AMEBASTATIC ZONE (IN DILUTIONS)	INCUBATION PERIOD (IN HOURS)	BACTERIA		AMEBA		
					GRAM		CYTOPLASM M (MICRONS)*	NUCLEUS M (MICRONS)*	DIVISION
GROUP	KIND				NEGATIVE	POSITIVE			
Triphenyl-methane	Gentian violet	1: 200 >	< 1: 200	72	+++	+	28.4	6.9	++
	Malachite green	1: 400 >	< 1: 400	68	+++	+	25.2	6.8	++
	Brilliant green	1:1,600 >	< 1:1,600	48	+	+++	22.8	6.8	+
	Pyoktanin	1:2,000 >	< 1:2,000	36	+++	+	20.7	6.2	−
	Acid fuchsin	1: 400 >	< 1: 400	58	+	+++	21.3	6.8	+
	Basic fuchsin	1:1,600 >	< 1:1,600	62	+++	+	21.6	6.4	−
Acridine	Acriflavine	1: 200 >	< 1: 200	72	+	+++	24.9	6.9	++
Quinoneimine	Neutral red	−	−	24	+	+++	21.6	6.6	+
	Methylene blue	1: 800 >	< 1: 800	36	+	+++	20.1	6.1	−
	Safranin	−	−	24	++	++	23.3	6.0	+
	Brilliant cresol blue	1: 200 >	< 1: 200	36	+	+++	20.7	6.1	−
	Toluidine blue	1: 200 >	< 1: 200	36	+	+++	21.3	6.3	−
Azo	Bismarck brown	1: 800 >	< 1: 800	48	+++	+	23.6	6.7	+
Xanthene	Eosin B	1: 400 >	< 1: 400	48	+++	+	21.7	6.6	−
	Mercurochrome	1:6,400 >	< 1:6,400	48	−	+	24.6	6.7	−
Control		−	−	18-24	++	++	20.3	6.4	+

*The size of ameba was determined by dividing the sum total of length and width of the trophozoite by 2.

M (Mean) was computed by using the following formula $M = \frac{\Sigma \times f}{n}$ in which n indicates the number of samples (100) ×, the measurements of all samples, and f, the frequencies in which they appeared.

microorganisms were probably affected by these dyes. The size of amebas was likewise increased, though much less than in the case of the organisms exposed to gentian violet.

According to Churchman[9] dye mixtures, though inferior to any of the constituent dyes alone in bactericidal action, seem to enhance the bacteriostatic power to such an extent as to suspend the growth of the organisms over a long period. When washed cysts were first exposed to the action of a mixture of an equal volume of gentian violet and acriflavine (1:100 dilution each) at 37° C. for one hour and then inoculated into culture medium, the amebas were usually found to maintain growth as long as eight days without further transfer. It is suggested, therefore, that this procedure may serve as a means of maintaining cultures for several days. Koch claimed that though acriflavine is known to be a powerful amebicide as tested against *E. gingivalis* in vitro, it is not so effective an amebastatic agent as gentian violet.

The duration of exposure of the cysts to the action of gentian violet, acriflavine, and malachite green clearly indicated that, while the cysts were often cultivable after an exposure of as long as twenty-four hours, an optimum degree of amebastasis was obtainable by an exposure of one hour to these dyes. As was to be expected, the optimum amebastatic effect was obtainable at 37° C., and it was less at an exposure to 5° C. or 22° C.

Acid and basic fuchsins exhibited the amebastatic effect on the cysts of *E. histolytica* in the dilutions of 1:400 and 1:1,600, respectively. The growth of amebas was first observable in cultures at fifty-eight hours in the former and sixty-two hours in the latter at 37° C. The increase in the size of the organisms was, however, negligible. Furthermore, none of the cultures thus treated was found to maintain growth any longer than three days. Apparently, the bacteriostatic effect of these dyes was less than that secured by gentian violet, acriflavine, or malachite green.

Churchman[10] stated that, as a rule, acid dyes were known to be much weaker in bacteriostatic action than basic dyes. This fact may explain why a higher concentration was necessary to induce amebastasis in the case of acid fuchsin than in the case of basic fuchsin.

As will be seen from results recorded on Table II, the dyes exhibited a higher degree of toxicity upon trophozoites than upon the cysts of *E. histolytica*. This is undoubtedly due to the greater permeability of the walls of the trophozoites. With respect to the intensity of toxic action, the dyes were found to be as follows: mercurochrome exhibited the greatest toxicity to the trophozoites, while acriflavine and methylene blue showed less. Neutral red, safranin, and eosin B, on the other hand, were only slightly lethal to the trophozoites.

The manner in which the trophozoites responded to the exposure to dyes in vitro is of interest. At the outset, the defensive mechanism of the organism was at work as shown by a continual attempt on the part of the organism to expel the dye particles from its cytoplasm. This process usually required from a few minutes to one hour, at which time no dyes were detectable microscopically within the amebas. In the cultures, the process of gradual regeneration of the organisms became evident as time elapsed, and eventually normal growth was resumed by the organisms. The viability of trophozoites as affected by

the dyes depends, therefore, upon the rapidity with which the dyes were eliminated from the protoplasm of ameba. In the case of gentian violet, acid fuchsin, malachite green, brilliant green, and basic fuchsin, the organisms continued to grow in the presence of these dyes as long as forty-eight hours upon continued incubation at 37° C., while no growth was discernible when other dyes were used. This seemed to indicate that the action of these dyes was amebicidal rather than amebastatic. There were no appreciable degrees of amebastasis, and this was a decided contrast to the condition observed in the case of the encysted stage of the organism. As a matter of fact, the life of amebas in the majority of instances was not prolonged beyond twenty-four hours, and the organisms soon disintegrated either owing to the overgrowth of

TABLE II

EFFECT OF DYES ON TROPHOZOITES OF ENDAMEBA HISTOLYTICA IN VITRO

DYES		LETHAL DILUTION	SUB-LETHAL DILUTION USED	INCUBATION PERIOD (AT 37° C.)					
				24 HOURS		48 HOURS		72 HOURS	
GROUP	KIND			GROWTH	DIVISION	GROWTH	DIVISION	GROWTH	DIVISION
Triphenylmethane	Gentian violet	1: 10,000	1: 20,000	+++	+	+++	+	–	–
	Malachite green	1: 10,000	1: 20,000	+++	+	++	Trace	–	–
	Brilliant green	1: 8,000	1: 16,000	++++	+	++	Trace	–	–
	Pyoktanin	1: 8,000	1: 16,000	++	–	–	–	–	–
	Acid fuchsin	1: 400	1: 800	+++	+	++	Trace	–	–
	Basic fuchsin	1: 2,000	1: 4,000	+++	+	+	–	–	–
Acridine	Acriflavine	1: 40,000	1: 80,000	+++	+	–	–	–	–
Quinone-imine	Neutral red	1: 400	1: 800	+++	+	–	–	–	–
	Methylene blue	1: 20,000	1: 40,000	+++	+	–	–	–	–
	Safranin	1: 1,000	1: 2,000	++++	++	–	–	–	–
	Brilliant cresol blue	1: 8,000	1: 16,000	+++	+	–	–	–	–
	Toluidine blue	1: 10,000	1: 20,000	++	–	–	–	–	–
Azo	Bismarck brown	1: 4,000	1: 8,000	++++	+	–	–	–	–
Xanthene	Eosin B	1: 800	1: 1,600	++	–	–	–	–	–
	Mercurochrome	1:100,000	1:200,000	+++	+	–	–	–	–

the accompanying bacteria or to an excessive accumulation of their own metabolic products. In a few instances, the bacteriostatic effect of the dyes was made quite evident through the survival of the amebas for an additional twenty-four hours.

Observations on the development of increased resistance of dyes upon repeated exposures were carried out with the use of gentian violet, acid fuchsin, and malachite green. It was found that repeated exposures of the trophozoites to the action of these dyes in sublethal dilutions eventually created an apparent degree of relative resistance, which increased in proportion to the number of exposures. Thus, in eleven exposures of the trophozoites to 1:20,000 dilution of gentian violet, no dye particles were found to permeate the protoplasm of the

amebas, while sixteen exposures were necessary to establish the resistance in the case of 1:800 dilution of acid fuchsin and eighteen exposures were required in the case of 1:20,000 dilution of malachite green.

DISCUSSION

The effect of dyes on the growth of amebas in culture medium seems to depend on two possible factors as suggested by Simon[11] in his work on dyes and bacterial development: (1) the presence of suitable receptors in the organism, and (2) the ease with which the receptors taking up the dye recover their capacities to unite with the dye. Susceptibility and resistance of the organism apparently hinge on the degree of avidity of these receptors. Dye susceptibility may change by adaptation. Thus, it is possible to change a dye-susceptible strain to a dye-resistant one by repeatedly exposing the amebas to the action of dyes such as gentian violet, acid fuchsin, and malachite green. The amebicidal action of dye in question may, therefore, be interpreted as indicating the existence of corresponding receptors in organisms with which the dye may unite.

Accordng to Stearn and Stearn,[12] the maximum bacteriostasis is produced by the formation of the dye-protein compound. A protein is readily liberated from the combination, so that unless some other mechanism is operative in actually destroying the bacterial protein while the growth is inhibited by the dye, the organisms remain latent awaiting for the opportunity to resume the development. The same appears to hold true with the proteins of amebas as was illustrated by the use of gentian violet and acriflavine or a combination of the two. Koch stated that dyes belonging to the acridine group are most active against bacteria in protein solution. They would, therefore, be of particular value in a medium containing egg albumen.

Variations in size of amebas observed after the exposure of the organisms to the action of the dyes may be partly explained as the result of the diminution in the bacterial population in culture. So long as this influence persisted the size of amebas remained irregular, although the organisms were able to grow without further transfer. Once, however, the effect of the dyes had passed, the amebas resumed the normal dimensions and were soon crowded out by the overgrowth of bacteria in the culture medium. It may also be possible that certain strains of amebas are capable of proliferating in vitro irrespective of the accompanying bacteria. The question of an increased oxygen tension of the culture medium may likewise play a rôle in size variations of the organism.

Attempts have been made in the past to separate amebas in culture medium from the accompanying bacteria. The utilization of proper concentrations of chemicals to induce the growth of amebas and simultaneously to kill the accompanying bacteria has been tried out. The cultivation of the organisms under the conditions of strict anaerobiasis and the isolation of the organisms from bacteria-free liver abscesses have resulted in apparent failure. Though dyes alone may not be used as a means of securing a pure culture, at least they would be of help in inhibiting bacteria where other means of isolation are to be used. It is suggested, therefore, that by the use of a combination of these measures, one may arrive at a method by which the amebas thrive in pure culture.

SUMMARY

1. The action of high concentration of dyes on the cysts of *E. histolytica* was found to be amebicidal, but when diluted they exhibited amebastatic effect which varied in intensity with the kinds and concentrations of dyes used. Amebastasis was best shown by the use of gentian violet, acriflavine, and malachite green, and to a lesser extent by basic and acid fuchsins. Safranin and neutral red apparently failed to produce the amebastatic effect, while the remainder of the dyes exerted a more or less intermediate degree of amebastasis.

2. In the cultures where amebastasis was induced through the addition of suitable concentrations of gentian violet, acriflavine, and malachite green, there was a marked increase in the size of the organisms. This may be accounted for by the antagonistic effect of the dyes upon the accompanying bacteria. So long as the effect remained operative, the amebas in cultures were able to thrive without transfer.

3. An optimum amebastasis was obtained by exposing washed cysts to the action of the dyes or of a mixture of gentian violet and acriflavine for one hour at 37° C., prior to inoculation into culture medium. In these circumstances, the lag period was prolonged, but the development thereafter was remarkably luxuriant. It is suggested that this procedure may serve as a means of maintaining cultures for several days. The action of dyes on the trophozoites of *E. histolytica*, on the other hand, was as a rule, amebicidal rather than amebastatic.

4. By repeated exposures of the culture of amebas to dyes such as gentian violet, acid fuchsin, and malachite green in proper concentrations, it was possible to increase the resistance of the amebas to these dyes. Significant increase of the resistance was secured by eleven successive daily exposures to 1:20,000 dilution of gentian violet, upon sixteen exposures to 1:800 dilution of acid fuchsin, and upon eighteen exposures to 1:20,000 dilution of malachite green.

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STUDIES ON THE EFFECT OF A HIGH-SULPHUR LOW-CARBOHYDRATE DIET IN CHRONIC ARTHRITIS*

J. C. Forbes, M.A., Ph.D., R. C. Neale, O. L. Hite, M.D.,
D. B. Armistead, M.D., and S. L. Rucker, M.D., Richmond, Va.

IT HAS been shown in a previous publication (Forbes and Neale[1]), that indoluria is usually present in patients with chronic arthritis, pellagra, congestive heart failure, toxemia of pregnancy, diabetes mellitus, tuberculosis, and severe lobar pneumonia. Furthermore, the indoluria more or less parallels the clinical condition, disappearing with marked improvement.

Indole may arise from any focus of infection in the body. Since in so many cases of chronic arthritis no focus of infection can be demonstrated, one is almost forced to believe that (in these cases) the origin of the indole is the decomposition of tryptophane in the intestinal tract, the indole being either formed in greater amounts than normal or allowed to pass through the liver undetoxified.

Indole is normally detoxified in the body by being converted into indican (potassium indoxyl sulphate). Therefore, sulphur is necessary for indole detoxification. Evidence that there may be a sulphur deficiency in chronic arthritis has been presented by Sullivan and Hess[2] and Argy,[3] who have found a lowered sulphur content of the finger nails in this condition. Couturat[4] has shown that the sulphur content of the articular cartilage is low in patients with chronic arthritis. Furthermore, various sulphur preparations have been used from time to time in the treatment of arthritis, and in many cases have led to clinical improvement. With these facts in mind, it occurred to us that indoluria might be evidence of impairment of liver detoxification resulting from a sulphur deficiency in the liver.

Thus, it seemed logical to give to arthritic patients a diet having a high proportion of sulphur amino acids and a low carbohydrate content. In addition to taking the place of proteins, thereby limiting the amount of sulphur ingested, there is evidence that carbohydrates in large amounts may have a direct detrimental effect. For example, Fletcher[5] produced in a case of arthritis an acute exacerbation of the condition by freely feeding carbohydrates. We have obtained similar evidence indicating the harmful effect of a high carbohydrate diet.

The importance of the diet in the treatment of chronic arthritis has been emphasized by a number of investigators, and the diets recommended are as a rule low in carbohydrates. Other factors are also stressed. Fletcher and Graham[6] feel that the vitamin B content is an important factor, since it improves the tone of the intestinal tract and prevents stasis of its contents. Pemberton[7]

*From the Departments of Biochemistry and Medicine, Medical College of Virginia.
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emphasizes the importance of a balanced, low-calorie low-carbohydrate diet. Cecil[8] recommends a low carbohydrate diet in conjunction with vaccines and other forms of therapy. Langstroth[9] has obtained from statistical studies a definite correlation between the incidence of a number of diseases, including chronic arthritis, and the amount of protective foods or vitamins in the diet. The incidence of these diseases was greatest in those individuals consuming the smallest amount of protective foods. Basing his therapy on these findings, Langstroth fed a low-carbohydrate high-vitamin diet to patients with chronic arthritis with gratifying results.

The diet used in the work here reported agrees to a considerable extent with those employed by the above investigators, having in common with them a low carbohydrate intake. However, we made no attempt to decrease the caloric intake; on the contrary, in all apparently undernourished patients the diet was kept at as high an energy level as feasible by the administration of readily digestible fats such as cream and butter. Furthermore, the content of sulphur amino acids was quite high.

In the cases here reported, treatment was confined to dietary control, together with the use of cathartics when necessary to obtain good elimination. The majority of patients on this diet improved markedly. Coincident with improvement, there was a decrease in the indole elimination, and finally its complete disappearance from the urine. Although the best results were obtained in the treatment of rheumatoid arthritis, some typical cases of hypertrophic arthritis also showed definite improvement. This was true even in one case where repeated examination of the urine failed to show the presence of indole.

The diet used by us is as follows:

Meats: At least one large portion of meat, such as chicken, veal, beef, fish, etc., daily in addition to from three to five eggs, and cheese in liberal amounts.

Vegetables: Five per cent vegetables such as lettuce, celery, spinach, cucumbers, cauliflower, and broccoli, together with small amounts of 10 per cent vegetables such as beets and carrots. The starchy foods such as corn, rice, potatoes, etc., are entirely eliminated.

Fruits: Two large oranges, tomatoes, or one grapefruit or its equivalent daily.

Sweets: All pastries, pies, cake and sweets in general are eliminated.

Bread: Bread is limited to three slices of toast a day.

Milk: At least one quart of milk or buttermilk daily, and butter in liberal amounts, especially to undernourished patients.

Alcohol: Alcoholic liquors in any form are strictly avoided.

Table I gives, in abbreviated form, the results of the determination of urinary indole on patients with different types of arthritis and their response to treatment.

Table II shows the changes in urinary indole during treatment in a few typical cases. The results indicate a fairly definite correlation between indoluria and the clinical condition. For instance, in the case of Mr. Ne. it will be seen that on discontinuing his diet for several weeks not only did his arthritic pains return but indole reappeared in the urine. Shortly after resuming the diet he became free from symptoms and his indoluria disappeared.

It is not possible from our results to state whether the beneficial effects obtained are due to the increased sulphur content of the diet, or to the increased vitamin B content, or to the diminished carbohydrate intake. It is

TABLE I

DIAGNOSIS	NUMBER OF PATIENTS	NUMBER WITH INDOLURIA	NUMBER WITH NEG. INDOLE OR TRACE	NUMBER TREATED	NUMBER MARKEDLY IMPROVED	NUMBER SLIGHTLY IMPROVED	NUMBER NOT IMPROVED
Rheumatoid arthritis	24	22	2	22	20	0	2
Hypertrophic arthritis	13	10	3	8	5	1	2
Mixed arthritis	2	2	0	2	1	0	1

TABLE II

PATIENT	DIAGNOSIS	DATE	INDOLE	CONDITION
Mr. El.	Hypertrophic arthritis	4/21	1.7	Suffering considerably
		6/ 2	1.3	Unchanged
		6/ 5	3.0	Unchanged
		6/ 6		Placed on "arthritis diet"
		7/10	Trace	Stronger, less pain
		7/31	Neg.	Considerably improved
		9/18	0.2	Few pains following recent exposure
Mrs. Ar.	Rheumatoid arthritis	6/ 7	0.7	In considerable pain
		6/ 8	0.5	Unchanged
		6/ 9		Placed on "arthritis diet"
		6/19		Markedly improved
		6/23	Neg.	Free from symptoms.
Mrs. Gr.	Hypertrophic arthritis	6/20	0.7	Suffering considerably
		6/21	0.9	Unchanged
		6/22	0.5	Unchanged
		6/23		Placed on "arthritis diet"
		6/28	Neg.	Improvement remarkable
		7/11	0.3	Some reappearance of pains following laxity with diet
		7/18	Trace	Much better
		7/31	Neg.	Some pains present following recent exposure to cold and damp
		8/ 7		Pain moderately returned following temporary discontinuance of diet
Mr. Ne.	Rheumatoid arthritis	6/23	1.0	Pain very severe
		6/25	0.7	Unchanged
		6/26	1.0	Unchanged
		6/27		Pain more severe, placed on "arthritis diet"
		6/28	0.8	Feeling better
		6/30	0.7	Somewhat improved
		7/ 5	0.5	Some improvement
		7/ 6	0.1	No pain
		7/ 8	1.4	Reappearance of pain, cause unknown
		7/18	0.6	Pain slight
		7/21	0.6	Pain slight
		7/27	Trace	In good condition
		7/29	Neg.	Free from pain
		8/24	Neg.	Continued good
		10/ 1		Diet discontinued
		10/15	1.2	Pains returned, rather severe
		10/16	1.1	Unchanged
		10/17	0.8	Unchanged, returned to diet
		11/ 6	0.3	Much better, but some pain present
		12/ 6	Neg.	In excellent condition, no pain

probable that all three may be contributing factors. Since sulphur is necessary for indole detoxification, and since indoluria disappears with improvement, it is reasonable to conclude that the increased sulphur content of the diet is a factor in bringing about the clinical improvement. Furthermore, we have produced typical arthritic changes in the joints of rabbits by the injection of small amounts of indole into the joint cavity. This work will be published in detail later. The importance of vitamin B through its effect on intestinal tone and function is obvious.

The results presented here do not eliminate the possibility that chronic arthritis arises from foci of infection in the body. However, there is possibly always putrefaction in the intestinal tract of the adult, with absorption of some of the products. If the liver were unable to detoxify these substances, it would seem natural to believe that they could produce the same general results as would arise from the presence in the system of these putrefactive products resulting from foci of infection. Furthermore, if the liver allows indole to pass without detoxification, it is likely that other known or unknown toxic substances may also pass through unchanged, and play important rôles in the etiology of chronic arthritis. If these assumptions are correct, then a study of the various factors influencing the detoxifying power of the liver may throw some light on the etiology of the disease and the means for its prevention. Work is being done on this at the present time.

SUMMARY

Observations have been made on the effects of a high-protein low-carbohydrate diet on the clinical course of patients with chronic arthritis, together with studies of the accompanying changes in urinary indole. Definite improvement resulted in the majority of cases, the best results being obtained in those patients with rheumatoid arthritis. Especially good results were obtained in young individuals in early stages of the disease. Coincident with improvement, the indoluria which almost invariably accompanied the disease in its active stages, diminished and finally disappeared. It is suggested that indole is causally related to chronic arthritis, and that diets rich in sulphur aid in the detoxification of indole with consequent clinical improvement in the condition.

The writers gratefully acknowledge the many helpful suggestions of Dr. William B. Porter and Dr. Frank L. Apperly during the prosecution of this work.

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THE BACTERICIDAL ACTION OF IRRADIATED OIL OF PINE ON HEMOLYTIC STREPTOCOCCUS*

FRANKLIN A. STEVENS, M.D., NEW YORK, N. Y.

TO DEMONSTRATE the bactericidal properties of irradiated oils, investigators have employed one of two methods. In the early reports, bacteria were seeded on agar plates which were inverted over dishes of irradiated oil, previously exposed to ultraviolet light, so that the bacteria were exposed to the vapor from the oils during incubation. In the more recent studies of Ross,[1] Thompson and Sheard,[2] and of Sears and Black,[3] the bacteria were mixed with irradiated lanolin, petrolatum or mineral oil. Frequent cultures of these mixtures were made and the results of these cultures compared with those on mixtures of the bacteria with oils which were not irradiated. In most of these investigations the oils and vapors from the oils have been found weakly germicidal. This germicidal property has been attributed by some to secondary radiation from the irradiated oil, but the recent studies of Harris, Bunker and Milas[4] show that the bacteria are probably killed by peroxides formed during the irradiation of the oil. Their conclusions are based on the facts that the bacteria are not killed if quartz plates are inserted between the surface of the oil and the plate seeded with bacteria, that organic peroxides are found in vapor from irradiated oils, and that the bactericidal effect of the vapor is proportional to the increased peroxide content of the oil occurring during its irradiation.

No studies of the effect of irradiation with ultraviolet light on the bactericidal properties of essential oils have so far been found in the literature. Since the animal, vegetable and mineral oils which have been investigated have not been bactericidal previous to irradiation, and have acquired germicidal properties subsequent only to exposure to ultraviolet light, the study of this acquired bactericidal effect is simpler than a similar investigation of essential oils. Many of these essential oils are normally germicidal and the vapor from them kills bacteria seeded on agar plates inverted over dishes of oil in the incubator. Neither of the methods successfully employed in demonstrating the bactericidal properties of the irradiated oil previously studied and reported in the literature were sufficiently accurate and quantitative to satisfactorily show that an irradiated essential oil was more bactericidal than the normal oil. In searching for a quantitative method of studying the bactericidal qualities of the normal oils, it was found that if a given quantity of oil was agitated with salt solution and after eighteen hours if the salt solution was withdrawn from beneath the layer of oil, this salt solution was not only bactericidal but also in repeated experiments would kill the bacteria in a fixed quantity of broth culture within a certain limit of time. The bactericidal qualities of this salt solution were so

*From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital.

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constant with the same oil in different experiments that this method was employed for comparing the germicidal qualities of normal and irradiated oils. Although the effect of irradiation on several oils was studied, the experiments given in detail here are those done with oil of pine. It has been found that irradiated oil of pine is more bactericidal than the normal oil.

EXPERIMENTAL

The Bactericidal Action of Salt Solution Vigorously Agitated With Essential Oils.—As noted in the preceding paragraphs, one of the difficulties in determining the bactericidal effects of irradiated essential oils was the accurate estimation of the difference between the bactericidal property of the normal oils and of oils which had been irradiated with ultraviolet light. The observation that salt solution shaken with an essential oil or with the active principle of an oil retained sufficient of the oil in solution to be bactericidal, solved this difficulty, inasmuch as it permitted an accurate determination of the bactericidal property of each sample of normal oil previous to irradiation. Salt solutions which had been shaken with essential oils and separated either by standing or by centrifugation were bactericidal. The germicidal action of these solutions, however, varied greatly. Two factors entered into these variations. First, lots of the same oil from different manufacturers varied greatly in their bactericidal quality against the same bacterium. Second, while some oils were universally bactericidal and other oils quite inert, some were more bactericidal for one variety of bacterium than for another. After a preliminary study of these variations with different oils and with different bacteria experiments were carried out with the same lot of oil and the same bacterium. In these later experiments the only variable was the effect of irradiation on the oil.

Exper. I. The Bactericidal Action of Salt Solution Previously Shaken With Essential Oils.—Two hundred cubic centimeters of sterile physiologic salt solution were shaken vigorously in rubber capped bottles with 2 c.c. of oils of pine, orange, eucalyptus, thyme and peppermint, and with linalool, eugenol, isoeugenol and rhodinol. These mixtures were allowed to stand two days until the oils had separated from the salt solution. Cultures of pneumococcus I, *Staphylococcus aureus, Streptococcus viridans, Streptococcus hemolyticus,* Friedländer bacillus, *Bacillus lepisepticum,* and *Micrococcus catarrhalis* were grown eighteen hours in broth containing 0.1 per cent dextrose. Nine cubic centimeters of each of these cultures were centrifuged and the sedimented bacteria were suspended in 3 c.c. of each of the salt solutions previously shaken with the different oils. Cultures were made from these suspensions on blood agar plates at frequent intervals to determine the lengths of time required to kill the bacteria. The limits of viability for these various combinations of bacteria and oils are arranged in Table I. In this table (Table I and the three succeeding Tables II, III and IV), the intervals elapsing between the time the bacteria were mixed with the salt solutions and the time at which they were killed have been indicated by the letter D followed by the elapsed time in minutes or hours. If the bacteria were living when cultures were discontinued, the letter L has been used with the interval at which the last culture was made. In a few instances S with the time interval has been used to indicate scant growth.

The Exhaustion of the Bactericidal Activity of Salt Solution Through Contact With Bacteria: The object of the preceding experiments was to show that sufficient oil remained either in solution or in suspension in salt solution agitated with it to render the salt solution bactericidal. No attempt was made to standardize the number of bacteria exposed to given amounts of salt solution, so that the results obtained give only approximate information concerning the comparative bactericidal qualities of the various oils. It was observed that heavy cultures were killed more slowly than lighter growths if exposed to the same quantity of the same salt solution. Following this observation, experiments were

TABLE I

THE BACTERICIDAL EFFECT ON DIFFERENT BACTERIA OF SALT SOLUTION PREVIOUSLY AGITATED WITH ESSENTIAL OILS

	OIL OF PINE	OIL OF ORANGE (SWEET)	OIL OF EUCALYPTUS	OIL OF THYME	OIL OF PEPPERMINT	LINALOOL	EUGENOL	ISOEUGENOL	RHODINOL
Staphylococcus aureus	D 20 hr.	D 24 hr.	L 48 hr.	L 24 hr.	L 48 hr.	D 24 hr.	D 30 min.	D 60 min.	L 48 hr.
Hemolytic streptococcus	D 1 min.	D 26 hr.	L 30 hr.	D 3 hr.	D 90 min	D 1 min	D 1 min.	D 1 min.	D 10 hr.
Streptococcus viridans	D 60 min.		Irregular	D 4 hr.	D 10 hr.	D 2 hr.	D 1 min.	D 1 min.	D 3 hr.
Pneumococcus (I)	D 20 min.		D 10 min.	L 24 hr.	D 24 hr.	D 20 min.	D 1 min.	D 1 min.	D 3 hr.
Micrococcus Catarrhalis	D 1 min.	D 5 hr.	Irregular	D 2 hr.	D 5 hr.	D 1 min.	D 1 min.	D 1 min.	D 10 min.
Friedlander bacillus	D 1 min.		Irregular	D 20 min.	D 24 hr.	D 1 min.	D 1 min	D 1 min.	D 30 min.
Bacillus lepisepticum	D 1 min.	L 24 hr.	D 20 min.	D 3 min.	D 10 min.	D 1 min.	D 1 min.	D 1 min.	D 10 min.

TABLE II

THE BACTERICIDAL PROPERTY OF SALT SOLUTIONS ON SUCCESSIVE EXPOSURES TO SUSPENSIONS OF BACTERIA (HEMOLYTIC STREPTOCOCCUS)

TUBE	SALT SOLUTION SHAKEN WITH LINALOOL	SALT SOLUTION SHAKEN WITH EUGENOL	SALT SOLUTION SHAKEN WITH ISOEUGENOL	SALT SOLUTION SHAKEN WITH OIL OF PINE
I	D 1 min.	D 1 min.	D 1 min.	D 1 min.
II	D 3 min.	D 8 min.	D 5 min.	D 4 min.
III	S 10 min.	L 20 min.	D 20 min.	D 15 min.
IV	L 20 min.	L 20 min.	S 20 min.	L 20 min.

undertaken to discover if the bactericidal effect of salt solution previously agitated with oil of pine, and with eugenol, isoeugenol or linalool, could be exhausted by repeatedly suspending hemolytic streptococcus in the same solution.

Exper. II. The Exhaustion of the Bactericidal Qualities of the Salt Solution.—Two cubic centimeters of linalool, eugenol, and of isoeugenol, and 20 c.c. of oil of pine were shaken vigorously with 100 c.c. of sterile salt solution and allowed to layer by standing. Cultures of an hemolytic streptococcus recovered from erysipelas (E 37) were grown for twenty-four hours. Nine cubic centimeter lots of this culture were centrifuged and the supernatant fluid was removed from the sediment. Three cubic centimeters of salt solution previously agitated with one of the oils were pipetted onto the sediment in one of these tubes. This

TABLE III

COMPARISON OF THE BACTERICIDAL EFFECT OF NORMAL AND OF IRRADIATED OIL OF PINE ON HEMOLYTIC STREPTOCOCCUS

DILUTION OF SALT IN RELATION TO OIL		1 TO 5		1 TO 10		1 TO 15		1 TO 20		1 TO 25		1 TO 30	
SAMPLE OF OIL	DURATION OF IRRADIATION OF OIL	IRRAD.	NORMAL	IRRAD.	NORMAL	IRRAD.	NORMAL	IRRAD.	NORMAL	IRRAD.	NORMAL	IRRAD.	NORMAL
A	1 hour	D 18 hr.	L 18 hr.										
B	3 hours	D 10 min.	D 10 min.	D 5 hr.	L 5 hr.	D 24 hr.	L 24 hr.	L 24 hr.	L 24 hr.				
B	3 hours			D 2 hr.	D 4 hr.			D 18 hr.	L 18 hr.				
B	3 hours	D 1 min.	D 1 min.	D 2 hr.	D 3 hr.	D 18 hr.	L 18 hr.	D 18 hr.	L 18 hr.			L 18 hr.	L 18 hr.
B	3 hours	D 2 hr.	D 3 hr.										
B	3 hours			D 8 hr.	L 8 hr.	D 24 hr.	L 24 hr.	S 24 hr.	L 24 hr.	L 24 hr.	L 24 hr.		

tube was agitated and cultured at intervals of one minute for twenty minutes. The suspension was then centrifuged and the supernatant salt solution was transferred to a second tube. This second suspension was cultured at minute intervals up to twenty minutes when, after centrifuging, the salt solution was transferred to a third then to a fourth tube. This experiment was repeated with salt solutions previously shaken with each of the four oils. The lengths of time during which the bacteria lived in each of the four successive tubes are arranged in Table II.

The Increased Bactericidal Qualities of Irradiated Oil of Pine for Hemolytic Streptococcus: In the first experiment, it was found that salt solution shaken with various essential oils was bactericidal but that the effect varied with different oils, with different samples of an oil obtained from different manufacturers and with the bacterium which was used in testing for the bactericidal effect. Many variables ordinarily entered into each experiment, and to demonstrate the increased bactericidal effect of oil which had been exposed to ultraviolet light, it was necessary to eliminate all variant factors except one, that of irradiation with ultraviolet light. In the following experiments this elimination

TABLE IV

ADSORPTION OF PEROXIDES ON HEMOLYTIC STREPTOCOCCUS FROM SALT SOLUTION AGITATED WITH IRRADIATED OIL OF PINE

	STAIN A	STAIN B	STAIN C	STAIN D
Peroxides in salt solution	++++	++++	++++	++++
Peroxides in salt solution after contact with bacteria	++	+++	+	++
Peroxide test with sedimented bacteria	++	++	++	++
Peroxide test with normal culture sediment	–	–	–	–

was accomplished by using the same sample of oil and the same culture of hemolytic streptococcus in each phase of the experiment. Oil of pine was tested both before and after irradiation with hemolytic streptococcus. Since the normal oil imparted bactericidal qualities to salt solution, it has been necessary to study the bactericidal effect of various dilutions of salt solution shaken with the oil, and to study the bactericidal effects of the salt solution shaken with irradiated oil in the range of dilutions just above that at which the "normal oil" solution was bactericidal.

Exper. III. The Increase in Bactericidal Effect of Oil of Pine Subsequent to Irradiation With Ultraviolet Light.—Two samples of oil from different manufacturers were employed in the following experiments. Sample A was normally less bactericidal than sample B. In most experiments with the irradiated oils, the oil was exposed for three hours in a large flat glass vessel to a quartz lamp at a distance of 30 inches. In each experiment, 40 c.c. of both irradiated and normal oil were shaken equally long and with the same vigor with 200 c.c. of sterile salt solution. After standing eighteen hours, the salt solutions were withdrawn from beneath the supernatant oil. Eighteen-hour cultures of hemolytic streptococcus (S 37) were used in each experiment. Nine cubic centimeter quantities of the culture were centrifuged and the sedimented bacteria were

mixed with different dilutions of the salt solution which had been in contact with the oil. Cultures were made on these suspensions at frequent intervals to determine the time limits of the viability of the bacteria in each solution. The conditions under which the normal control series were done were identical with those for the irradiated oil. The irradiation of the oil was the only variable. In Table III the dilutions of salt solution have been expressed as the relative proportions between the salt solution in the final dilution and the quantity of oil used in the original dilution from which these final dilutions were made.

The Adherence to Bacteria of Peroxidic Substance Extracted in Salt Solution From Irradiated Oil of Pine: The observation that vapors arising from the surface of oils, previously irradiated with ultraviolet light, inhibit the growth of bacteria has been reported frequently. So far the mechanism of this inhibiting effect has not been satisfactorily explained. The inhibition to growth was at one time attributed to secondary waves of radiant energy, but numerous experiments have been devised by various investigators proving this explanation unsatisfactory. Investigations by Harris, Bunker and Milas[4] have shown that volatile peroxides are formed in irradiated oils, and that the "bactericidal" property of the vapor from an oil is proportional to the increase in peroxide content of the oil occurring during the period in which it is exposed to ultraviolet light. In experiments undertaken in this laboratory,[5] it was found that both peroxides and aldehydes result from irradiation of oils but, so far, proof that the peroxides or the aldehydes account for the inhibition of bacterial growth has not been obtained. On the other hand, it has been found that the peroxides formed in oil of pine, when it is irradiated, can be extracted with salt solution and adhere to bacteria suspended in the salt solution in which these peroxides have been extracted. The adherence of these peroxides to the bacteria is demonstrated in the following experiment.

Exper. IV. The Extraction of Peroxides From Salt Solution by Bacteria.— The peroxides, formed in oil of pine as a result of irradiation, deliver oxygen slowly. In this respect they resemble the peroxides investigated by Harris, Bunker and Milas.[4] The oil has been tested directly both previous and subsequent to irradiation by adding 1 c.c. of N/100 HCl, 0.1 c.c. of a 5 per cent solution of KI and a few drops of starch solution. When these mixtures are shaken vigorously and then allowed to stand several hours, the unirradiated oil at the most develops a faint blue color, while the reaction with the irradiated oil is intense. Salt solution which has been shaken vigorously and separated from normal oil of pine usually has given a faint or negative test. Solutions separated after shaking with irradiated oil give strongly positive reactions. In the following experiments four strains of hemolytic streptococcus were grown eighteen hours, then 50 c.c. of each of these cultures were centrifuged. Salt solution previously agitated with oil of pine which had been irradiated previously was pipetted onto the sediments. Three cubic centimeters of the salt solution were mixed with the sediment from 50 c.c. of each of the cultures. The suspensions were agitated gently at intervals and centrifuged after six hours. One cubic centimeter of the salt solution which had not been in contact with bacteria, 1 c.c. of the supernatant solution which had been in contact with bacterial sediment, and the sediments themselves were tested for peroxides by the addition of the

quantities of acid, KI and starch used in testing oils directly. The peroxides in the salt solution were decreased after contact with streptococcus and the sediments gave positive tests. Control tubes of untreated bacterial sediment showed no peroxides.

SUMMARY AND CONCLUSIONS

In the preceding experiments, the observation that sterile salt solution which has been shaken vigorously with oil of pine retains enough of the oil either in suspension or in solution to be bactericidal has furnished a method by which the bactericidal properties of oils which have been irradiated could be compared with those of normal oils. The methods used in the past, both that of studying the bactericidal effects of vapors arising from the surface of the oils and that of mixing the bacteria directly with the oils, have proved unsatisfactory. In these experiments, in which the bactericidal qualities of oil of pine for hemolytic streptococcus were studied, it was found that salt solution agitated with irradiated oil of pine was more bactericidal than salt solution agitated with normal oil. The bactericidal quality of the salt solution was exhausted if bacteria were repeatedly suspended in the same solution. One chemical change has been observed in oils which have been irradiated, both the peroxides and the aldehydic content of the oils are increased. The peroxides formed during irradiation can be partially extracted from the oils in salt solution, and, although it has not been shown that these peroxides are bactericidal, bacteria exposed to these solutions adsorb and remove the peroxides from solution.

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RELATION OF BACTERIAL INFECTION TO LIVER INJURY*

Studies in Cinchophen Intoxication

Leo S. Radwin, M.D., and Max Lederer, M.D., Brooklyn, N. Y.

SINCE the publication of reports of poisoning by cinchophen, many attempts have been made to produce injury to the liver experimentally in animals by means of the drug.[1-8] The results of these investigations, however, have not been conclusive as to the rôle of the drug as a liver poison. Changes in the livers of animals which have been observed have been indefinite, not regularly reproducible, and unlike those reported in cases of human poisoning. Lehman and Hanzlik[1] emphasize that by the use of doses comparable to the therapeutic dose in human beings, no investigators have been able to produce in animals injury to the liver by cinchophen akin to the reported clinical picture.

In view of the unconvincing results from the simple administration of cinchophen to animals, it seems likely that where the drug is responsible for liver damage in man, there may be present some peculiar susceptibility of that individual to it. This susceptibility may be the result of previous injury to the liver, dietary irregularities, the influence of bacterial activity, etc. Working on a similar hypothesis, Reichle[2] first attempted to injure the liver by chloroform before giving cinchophen to white rats. However, he failed to find on autopsy any pathologic changes of importance. Lehman and Hanzlik[1] found that in rabbits, cinchophen did not increase the impairment of liver function previously caused by chloroform or phosphorus. In a previous work,[4] we found that incidental pulmonary infection in rats did not aggravate the toxic effects from cinchophen in massive doses, while a high carbohydrate diet served to minimize them. Infectious processes taking place in the body may be of great significance, however, in that they affect the liver first, and at times quite severely.[9-12] The part played by the liver as an organ of defense against foreign bodies of all types has been well reviewed by Gunn[13] and need not be taken up here.

An etiologic relationship between damage to liver tissue by hepatotoxins and the influence of bacterial organisms upon the liver has been demonstrated by Opie.[14] The latter's experiments indicate that the activity of an hepatic poison when used in doses which alone produce little change, may be so intensified by bacterial infection as to cause destruction of almost the entire hepatic parenchyma. Even bacteria such as colon bacilli, which have relatively little pathogenicity for normal animals, become virulent when the liver is injured by a poison such as chloroform or phosphorus. He showed that the resulting liver

*From the Pediatric Research Laboratory and the Department of Pathology of the Jewish Hospital of Brooklyn.

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changes could not be produced by either the hepatic poison or the bacteria alone. Most important, by the combination of chloroform and colon bacilli, he was able to reproduce in dogs a lesion closely resembling that of acute yellow atrophy in man. He also succeeded in demonstrating the same lesion in another animal, the rabbit, using a different drug, phosphorus, and a different organism, the streptococcus.

In this investigation, we have confined ourselves to a consideration of the sensitizing effect upon the cells of the liver, of living bacteria circulating in the blood stream and possibly localizing in the gallbladder or liver. More than forty years ago, Blachstein and Welch[15] demonstrated the persistence of colon or typhoid bacilli in the gallbladders of rabbits months or years after their injection into the general circulation. We have therefore attempted, by means of the coincident administration of cinchophen and living bacteria, to further study the deleterious action of the drug upon the liver.

METHOD

Only adult rabbits were used. Cinchophen was administered as a 10 per cent solution of the sodium salt, with beta eucain lactate. The drug was given daily, or every other day, by divided injections into the shaved thigh. Bacterial injections were given by way of the ear vein, twenty-four hour broth cultures being used. The injection of bacteria was usually begun several days after the onset of drug administration. At the end of an experiment, the animals which survived were put to death by an intravenous injection of air.

MINIMAL LETHAL DOSE

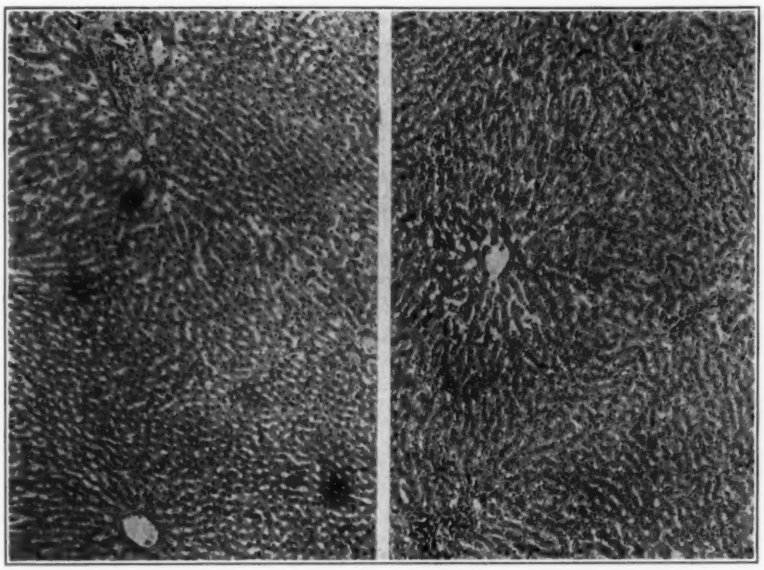
By means of graduated doses administered to different sets of rabbits, the minimal lethal dose of the cinchophen preparation was found to be 0.7 gm. of cinchophen per kilogram of body weight.

Autopsy findings in all the animals that died as a result of this procedure were essentially the same. There was congestion and hemorrhage in all the viscera. In the liver there was retention of bile in the cells in the region of the central veins and in the reticulo-endothelial cells lining the sinusoids. In the spleen, the macrophages and fixed histocytes were filled with altered blood pigment.

STREPTOCOCCUS

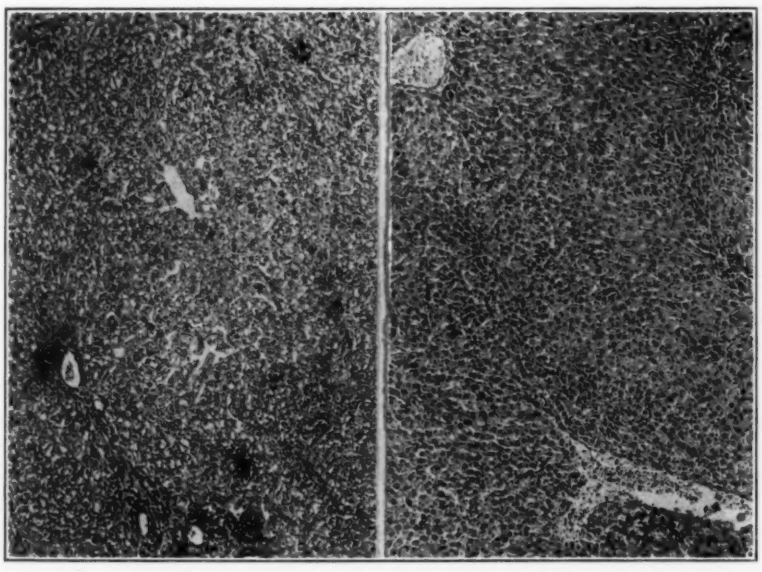
A large number of reported cases of cinchophen poisoning have been in patients suffering from either chronic cholecystitis or chronic arthritis. The streptococcus is the organism most commonly found in inflammation of the biliary passages[16] or in chronic arthritis.[17] A number of experiments were therefore performed, in each of which, animals were given cinchophen in large doses and also different strains of streptococcus. At the same time different sets of control animals were given either large doses of cinchophen alone, or the respective bacteria alone. The experiments were mostly repetitions in whole or in part of each other, varying in dose or strain of bacteria. Tables I and II describe the details of several typical experiments and give briefly the pathologic findings on autopsy.

From the tables it can be seen that attempts to modify the action of cinchophen by means of the streptococcus were unsuccessful. The liver changes re-



A. *B.*

Fig. 1.—*A*. Rabbit 39. Massive doses of cinchophen. Liver essentially normal. *B*. Rabbit 42. Cinchophen and *Streptococcus viridans*. Architecture undisturbed. Mild granular degeneration. Increase in polymorphonuclear leucocytes.



A. *B.*

Fig. 2.—*A*. Rabbit 47. *Streptococcus hemolyticus*. Granular and hydropic degeneration. Congestion. Miliary collections of polymorphonuclear leucocytes. *B* Rabbit 46. Cinchophen and *Streptococcus hemolyticus*. Architecture undisturbed. Congestion. Increase in number of polymorphonuclear leucocytes. Changes less severe than in Animal 47.

sulting from the combined use of cinchophen and various strains of streptococcus were similar to, and in some cases, even less severe than those observed after the

administration of comparable doses of the same strains of bacteria alone. Similar findings were noted in the other experiments involving the use of larger or smaller doses of different strains of streptococcus with cinchophen. Cinchophen alone produced only some congestion and capillary dilatation in the liver.

TABLE I

SUMMARY OF RESULTS FROM THE COMBINED INJECTION OF CINCHOPHEN (0.3 TO 0.7 GM. PER KILO) AND STREPTOCOCCUS VIRIDANS (0.25 TO 1.5 C.C.)

	RABBIT	CINCHOPHEN INJECTIONS		NO. OF BACTERIAL INJECTIONS	RESULTS	AUTOPSY FINDINGS (LIVER)
		NO. OF INJ.	TOTAL DOSE PER KILO (GM.)			
Cinchophen	39	32	12.6	0	Died on 42nd day	Essentially Normal. Architecture undisturbed. Some capillary dilatation and congestion.
	43	20	5.9	0	Died on 23rd day	
Cinchophen and *Strept. viridans*	41	21	6.6	5	Died on 27th day	Architecture undisturbed. Mild granular degeneration. Reticulo-endothelial cells prominent. Increased number of polys in sinusoids and capillaries. One large abscess with surrounding reaction.
	42	34	13.7	11	Killed on 46th day	
Strept. viridans	44 45	0	0	11	Killed on 36th day	Findings similar to those in animals Nos. 41 and 42, but more severe and more widespread.

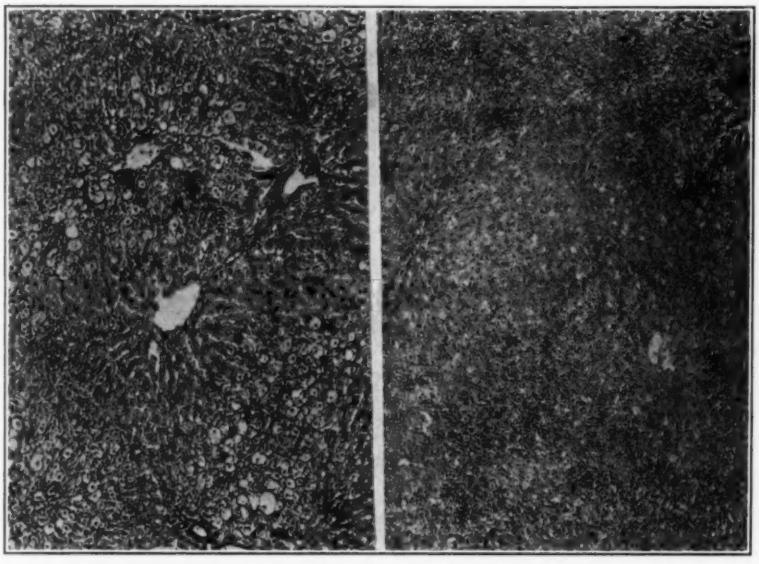
TABLE II

SUMMARY OF RESULTS FROM THE COMBINED INJECTION OF CINCHOPHEN (0.3 TO 0.5 GM. PER KILO) AND STREPTOCOCCUS HEMOLYTICUS (0.25 TO 0.5 C.C.)

	RABBIT	CINCHOPHEN INJECTIONS		NO. OF BACTERIAL INJECTIONS	RESULTS	AUTOPSY FINDINGS (LIVER)
		NO. OF INJ.	TOTAL DOSE PER KILO (GM.)			
Cinchophen and *Strept. hemolyticus*	46	18	4.2	3	Died on 22nd day	Moderate capillary dilatation. Increase in polys. Slight periportal round cell and fibrous tissue reaction.
	49	21	5.5	5	Died on 26th day	
Strept. hemolyticus	47 48	0	0	5	Killed on 26th day	Marked granular and some hydropic degeneration. Congestion and capillary dilatation. Increase of polys in blood vessels and miliary collections in parenchyma.

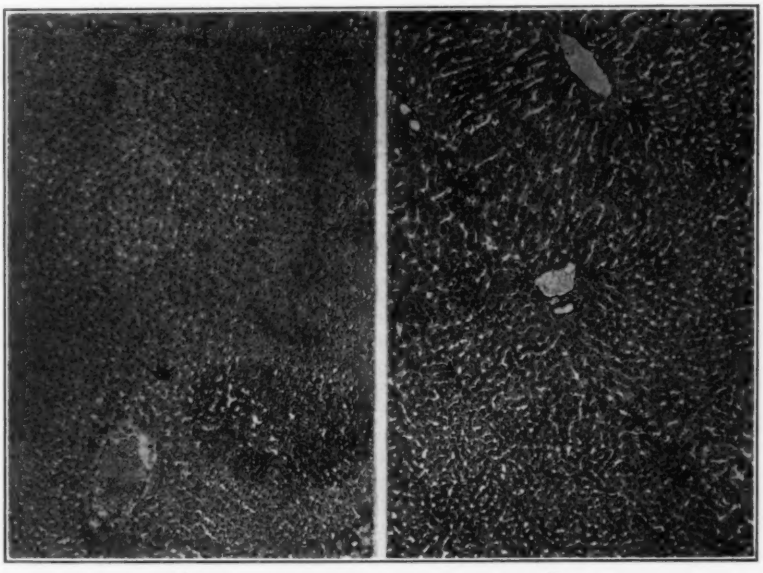
B. COLI

Most of Opie's observations[14] had been based upon the administration of colon bacilli to animals receiving chloroform. A study was therefore attempted of the effect of the administration of colon bacilli with cinchophen. At the same time, to permit a comparison between the two drugs, groups of animals were given the same strain of colon bacilli and chloroform. In the chloroform studies,



A. *B.*

Fig. 3.—*A*. Rabbit 61. Massive doses of chloroform. Architecture undisturbed. Fatty infiltration about central veins. The cells distal to this fatty layer are relatively intact. No hemorrhage. No necrosis of cells. *B*. Rabbit 24. Chloroform and *B. coli*. Extensive destruction of cells about the central veins. Marked hemorrhage. Cellular invasion. Only a narrow fringe of relatively intact cells remains about the portal structures.



A. *B.*

Fig. 4.—*A*. Rabbit 58. *B. coli*. Granular and fatty degeneration. Congestion. Abscess formation (lower right). *B*. Rabbit 15. Cinchophen and *B. coli*. Architecture intact. Capillary dilatation. Mild granular degeneration of cytoplasm of some of the cells. Lesion is less severe than in Animal 58.

Opie's technic was followed as closely as possible. The procedure in the main was as in the streptococcus groups. Animals were given various doses of colon bacilli, and large doses of cinchophen, or various doses of chloroform dissolved in paraffin oil. The cinchophen preparation was the same as that used before. In some experiments the bacteria were given with cinchophen or with chloroform, sixteen days after the previous administration of a single large dose of the same

TABLE III

RESULTS OF INJECTION OF CHLOROFORM (0.5 C.C. PER KILO) AND COLON BACILLI (0.5 C.C.)

	RABBIT	CHLOROFORM	B. COLI	RESULTS	AUTOPSY FINDINGS (LIVER)
		NO. OF INJ.	NO. OF INJ.		
Chloroform and colon bacilli	16 20	4 (daily)	1	Died on 5th day	Marked destruction of cells in two-thirds of the area about the central vein, with extensive hemorrhage throughout and areas of hyaline necrosis. Adjoining tissue foamy, with evidence of hydropic degeneration. Only a narrow rim of intact cells about portal structures. Poly and fibroblastic invasion. Extensive fatty infiltration.
Chloroform	51	6	0	Died on 9th day	Fatty infiltration of about one-third of area about the central vein. Remaining tissue to the portal spaces normal. No necrosis. No hemorrhage.
	61	10	0	Killed on 24th day	

TABLE IV

RESULTS OF INJECTION OF CINCHOPHEN (0.3 TO 0.5 GM. PER KILO) AND COLON BACILLI (0.25 TO 0.5 C.C.)

	RABBIT	CINCHOPHEN		B. COLI	RESULTS	AUTOPSY FINDINGS (LIVER)
		NO. OF INJ.	TOTAL DOSE PER KILO (GM.)	NO. OF INJ.		
Cinchophen and colon bacilli	23	7	2.2	1	Died on 15th day	Architecture undisturbed. Slight granular degeneration. Capillary dilatation. Focal collections of polys throughout parenchyma. Infected thrombus in blood vessel.
	19	10	3.5	2	Died on 24th day	
	15	10	3.5	2	Killed on 24th day	Many small abscesses. Pronounced fatty and granular degeneration. Congestion.
Colon bacilli	57 58	0	0	2	Killed on 24th day	

organism. As controls, groups of animals were given either the bacteria alone, cinchophen alone, or chloroform alone. Tables III, IV, and V describe several experiments in detail, which are typical of the many performed.

The liver changes repeatedly seen by us after giving chloroform alone, consisted essentially of fatty degeneration about the central veins. By the coincident administration of colon bacilli with chloroform, there was produced a lesion involving extreme destruction of liver tissue and resembling that of acute yellow atrophy. These findings coincided with those observed by Opie after a similar procedure.

In the many experiments in which cinchophen was given with colon bacilli, there was noted no difference between the liver changes caused by the combination of drug and microorganism, and those caused by bacteria alone. These changes consisted of marked congestion, either fatty or granular degeneration of the cytoplasm, or a combination of the two, and at times the presence of small miliary abscesses. The lesion in either case could be imputed only to the microorganisms.

TABLE V

RESULTS OF INJECTION OF COLON BACILLI AND CINCHOPHEN (0.3 TO 0.6 GM. PER KILO) OR CHLOROFORM (1.0 C.C. PER KILO) SIXTEEN DAYS AFTER THE PREVIOUS INJECTION OF 0.5 C.C. OF COLON BACILLI

	RABBIT	CINCHOPHEN		B. COLI		CHLOROFORM	RESULTS	AUTOPSY FINDINGS (LIVER)
		NO. OF INJ.	TOTAL DOSE PER KILO (GM.)	NO. OF INJ.	DOSE (C.C.)	NO. OF INJ.		
Chloroform and colon bacilli	2	0		2	0.1 -0.2	4	Died on 27th day	Tremendous liver destruction similar to that found in other animals given the combination of chloroform and colon bacilli, e.g., Nos. 16 and 20. (Table III.)
	24	0		2	0.1 -0.2	4	Died on 27th day	
	13	0		2	0.1 -0.2	4	Killed on 27th day	
Cinchophen and colon bacilli	14	11	3.5	6	0.05-0.25	0	Died on 39th day	Architecture undisturbed. Marked granular degeneration. Congestion. Capillary dilatation.
	22	19	8.5	9	0.05-0.25	0	Killed on 62nd day	
	62	20	9.2	9	0.05-0.25	0	Killed on 63rd day	

COMMENT

We have been able to corroborate Opie's findings that liver changes can be induced by the combined action of bacteria and an hepatic poison, which do not resemble the changes produced by either one alone. A lesion has been produced by the use of colon bacilli and chloroform together, which is not caused by chloroform alone even in massive doses, and which closely simulates that of acute yellow atrophy. Opie has brought about similar changes by the combined use of phosphorus and *Streptococcus hemolyticus*. None of the above changes assume the characteristics of those produced by either the microorganism or the chemical poison alone, but constitute an entirely different lesion, much more severe than is ordinarily encountered.

On the other hand, when cinchophen was given together with living bacteria, there was no modification of the reaction customarily caused by the bacteria. The effect was as though no hepatic poison had been employed. In this respect, therefore, cinchophen does not act as a liver poison such as is either chloroform or phosphorus. A clear-cut picture of liver destruction from the administration of cinchophen alone could not be demonstrated, nor has it been possible by our procedures to magnify the deleterious action of the drug upon the liver.

It has been the opinion of clinical observers that individuals suffering from biliary or hepatic infections are more prone to toxic disturbances from cincho-

phen. The introduction of colon bacilli into the circulation provides an infective agent which persists in the biliary passages for some time.[14] Although large doses of cinchophen were given to animals in whom microorganisms, including colon bacilli, had been repeatedly injected in this way over a long period of time, no liver lesions attributable to cinchophen were encountered.

In view of the convincing nature of some of the clinical reports, it is highly probable that cinchophen does at times exert a toxic influence upon the human liver. Unless the drug is limited in its toxicity to human beings, it should be possible to reproduce these effects in animals. This has not yet been accomplished. We have been able to show in this and in previous investigations[4] that cinchophen given in any dose does not produce an anatomic lesion in the livers of rats or rabbits. This fact, coupled with the rarity of authentic cases of poisoning, in the face of such an overwhelming number who have taken the drug in large doses, and over long periods of time without untoward effects, bespeaks a susceptibility to the drug which is not universal. On the other hand, relatively small doses of cinchophen have been employed in many of the reported cases of poisoning. In view of these facts, and of the apparently allergic nature of some of the reported symptoms,[18] it is not surprising that both clinical and experimental investigators have been led to consider the question one of idiosyncrasy or allergy. This stand has recently been taken by Short and Bauer[19] and by Quick.[20] The latter advances the interesting theory that liver necrosis, when it occurs, is a manifestation of the Arthus phenomenon.

As we stated earlier, it is not unlikely that the solution of this perplexing problem will lie in the demonstration, in individuals actually suffering from cinchophen poisoning, of a state of hypersensitivity of the cells of the liver, either because of an hereditary idiosyncrasy, or an acquired intolerance. Such a concept has also been proposed by Sulzberger.[21] An acquired intolerance may be due to a variety of unknown factors. If one of these is the action of microorganisms upon liver cells, it is clear that unless their effect is a most unusual one, the organisms involved are probably not of the common types, since cases of cinchophen poisoning are rare, while microorganisms are frequently present in the body. Moreover, it seems evident that the mere introduction of microorganisms into the circulation is not sufficient to so sensitize liver cells as to render them susceptible to injury by cinchophen. Further studies are being conducted along the lines which we have here suggested.

CONCLUSIONS

1. The lethal dose of cinchophen for the rabbit is 0.7 gm. per kilogram of body weight.

2. Further evidence is presented to indicate the profound manner in which the coincident administration of bacteria influences the action of an hepatic poison.

3. Cinchophen given in large doses produces no anatomic effect upon the rabbit liver.

4. Cinchophen given in large doses, in combination with colon bacilli, or with various strains of streptococcus, produces no anatomic lesion in the rabbit liver

other than that produced by the bacteria alone. The coincident administration of bacteria has failed to magnify the potentiality of cinchophen to induce damage to the rabbit liver.

5. The possibility of an unusual state of sensitization of the liver to the drug may be entertained in order to explain the clinical reports of cinchophen intoxication.

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LUNG ABSCESS*

A Clinical Consideration of 101 Cases

Harold C. Lueth, Ph.D., M.D., and Don C. Sutton, M.S., M.D., Chicago, Ill.

THERE is much confusion concerning the term lung abscess, so that four diagnostic standards were arbitrarily chosen for this study. They were: (a) aspiration of considerable amounts of pus followed in many instances by the localization of the cavity by the bronchoscope, (b) an x-ray film showing an irregular dense air shadow over circumscribed fluid, (c) the expectoration of large quantities of foul-smelling sputum particularly after a suggestive history, and (d) postmortem records. Some cases had only one of these features, while the majority had two or more. The present series was selected from a group of more than 160 patients discharged with a diagnosis lung abscess from the Cook County Hospital during the years 1933 to 1934. No case of tuberculous abscess was included in the group. Many small and early abscesses have been rejected for want of specific diagnostic points, and it is freely admitted this selection of cases tends to give a gloomier picture of the disease than is warranted. With the exception of three cases of multiple abscesses all were classified as solitary abscess.

ETIOLOGY

Incidence.—There were about 700 medical admissions for every case of lung abscess.

Age.—The number of lung abscesses increases by decades until the fifth, then it falls rapidly. Ages ranged from eleven days to sixty-six years. More than half the cases occurred in patients between thirty and fifty years. Mortality rates and incidence paralleled one another, so that about one-half the patients in each decade died (Fig. 1).

Sex.—The disease occurred more than three and a half times as often in men as in women (Table I).

Table I

Incidence of Sex and Color

	Sex	Improved	Same	Died	Total
White	Male	37	7	29	73
	Female	5	2	3	10
Colored	Male	7	1	6	14
	Female	2		2	4
Total		51	10	40	
Aggregate					101

*From the Department of Medicine, Northwestern University Medical School, and the Medical Division of Cook County Hospital.

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Color.—There were 83 white and 18 colored patients studied. Nearly 35 per cent of all medical admissions are colored, thus relatively more white than colored people have the condition.

Predisposing Causes.—Upper respiratory infections preceded 30 per cent of lung abscesses (Fig. 2). Two to six weeks, most commonly three, elapsed from the acute infection until signs of abscess were seen. No localizing sign

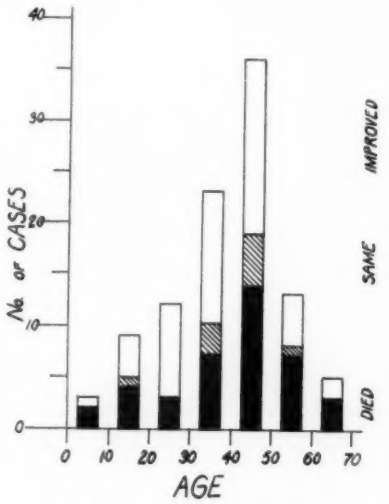

Fig. 1.—Age incidence.

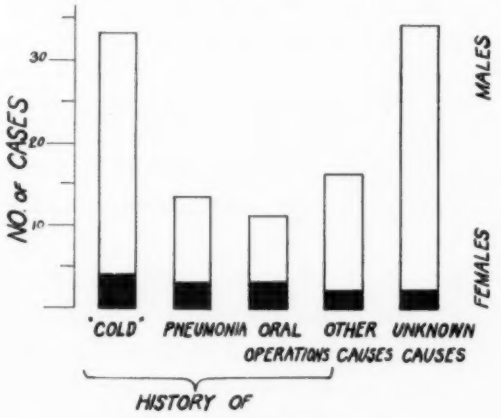

Fig. 2.—Predisposing causes.

or symptom of these infections indicated later abscess formation. Pneumonia preceded abscess in 13 per cent of all patients. The abscess always involved the same lobe or lobes as the pneumonia, being diagnosed after an interval of three to five months. Operations about the mouth and throat were responsible for 11 cases. Almost uniformly two and a half months elapsed between the operation and the onset of profuse expectoration. Miscellaneous causes were noted in 16 cases, while no definite etiologic factor could be assigned in 31 cases.

LOCATION OF THE ABSCESS

Abscesses occurred in every lobe of the lungs (Fig. 3). Eighty-seven were unilocular whose location was fixed by the x-ray or the bronchoscope. Either the lack of diagnostic plates or failure to use the bronchoscope left 11 undetermined. Location of abscesses was given in thirds of the lung fields as seen in the x-ray films, rather than the usual terminology, lobes of the lungs. Multiple abscesses were uniformly fatal.

SYMPTOMATOLOGY

Expectoration was by all odds the most common and significant complaint. It was present in 95 cases, and quite profuse in most of them. The color varied with the type and severity of infection, the ease of bronchial drainage and the mode of treatment. Hemoptysis was the chief symptom in 19 cases; 10

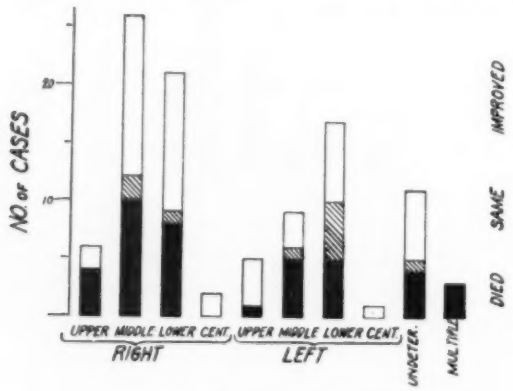

Fig. 3.—Location of abscess.

patients recovered while 9 died. Sudden massive hemorrhages were responsible for 2 deaths, and a third patient bled intermittently for seventy-two hours before he died.

The position of the abscess with respect to a bronchus frequently determined the nature of the symptoms. Early, with the formation of the abscess walls, there was usually an obstruction to a bronchus. A troublesome cough with scant thick secretion was common. Maturation of the abscess with softening of the bronchial plug followed. A sudden gush of putrid, bad-tasting material in the mouth after a slight cough indicated the beginning of drainage. In many cases this discharge of pus continued until the abscess became a cavity whose walls collapsed and healing took place by fibrosis. Intermittent expectoration of pus meant intermittent drainage. Persistently large amounts of expectoration were results of chronic abscess cavities.

Pain in the chest was a common symptom occurring in 55 cases. A moderately sharp pleural pain that radiated down the side of the chest, particularly down the midaxillary line, was a very frequent complaint. In a number

of cases confusion arose when there was pain over both chests; these patients had additional pathology on the side opposite the abscess. Neither central nor upper lobe abscesses caused pleural pain.

Loss of weight and strength was striking and was specifically mentioned in 42 cases. They were more dependent upon the duration of the disease than upon the etiology or location of the abscess.

Fever was present in almost every case. It began as a slight elevation in temperature either once or twice daily. An encapsulated abscess always showed the febrile changes of sepsis. The course of the fever was then determined by drainage. With rupture of an abscess and free drainage, the temperature fell to normal. Recrudescences meant partial obstructions and toxic absorptions. Dyspnea occurred infrequently. Other symptoms were recorded, but they were the results of complicating conditions.

PHYSICAL EXAMINATION

Three important findings of a lung abscess are: dullness, râles, and altered breath sounds (Table II). In 60 per cent of the entire group dullness to percussion was elicited. Râles were heard in 58 per cent, the fine moist type predominating. Nearly one-third of all patients had diminished breath sounds, 17 harsh to increased, 12 amphoric, and 11 bronchial. Other signs were found, but they were variable and inconstant. This is in accord with the studies of Norris and Landis,[1] who found signs of cavity in 8 patients from a group of 63.

TABLE II

PHYSICAL FINDINGS

		T. FREM.		PERCUSSION				BREATH-SOUND				OTHER FINDINGS		
		INCREASED	DECREASED	DULLNESS	FLATNESS	TYMPANY	CRACKED-POT	AMPHORIC	BRONCHIAL	INCREASED	DECREASED	WHISPERED PECTORILOQUY	FRICTION SOUND	RÂLES
Right	Central								1					2
	Upper	1		4			1	1	1					3
	Middle	1	5	20	3			3	3	7	9	1	1	17
	Lower	1	4	12	6			3	2	2	12	1	1	16
Left	Central										1			
	Upper	1	2	4		1			2	1	2	1		4
	Middle	1	1	6	3			3	1	4		1		6
	Lower	1	7	14	2			2	1	3	9	1	1	10
Total		6	19	60	14	1	1	12	11	17	33	5	3	58

In general the earlier the lesion the fewer the physical findings. Often areas of dullness were seen to increase in size, breath sounds became more suppressed and tactile fremitus decreased as the abscess appeared. Then following rupture, amphoric breathing, increased tactile fremitus, and loud moist râles were heard. All signs decreased with healing, although when a chronic cavity formed, they persisted unimpaired.

LABORATORY EXAMINATION

Urinary changes were those of fever. A moderate secondary anemia was discovered in those patients with chronic slight hemoptyses or with frank hemorrhages. The leucocyte count furnished an excellent prognostic guide. High leucocyte counts, 25,000 or more, early in the disease were generally favorable; low ones unfavorable.

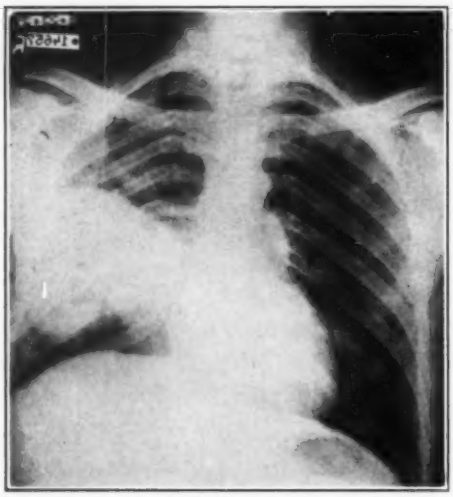

Fig. 4.

Fig. 5.

Fig. 4.—Large abscess in the middle third of the right lung prior to rupture.

Fig. 5.—Lateral view of the patient showing partial evacuation of pus with a fluid level.

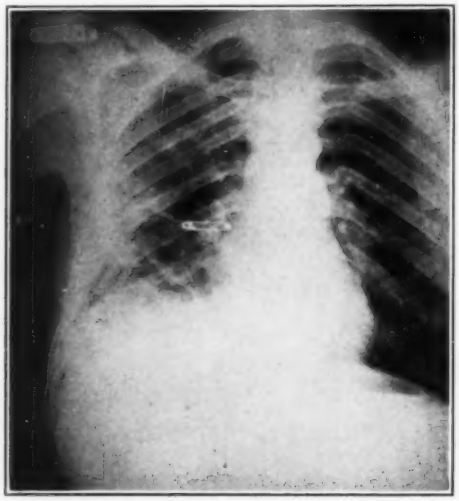

Fig. 6.—Same patient showing complete healing of the abscess.

ROENTGEN RAY EXAMINATION

A definite fluid accumulation within the parenchyma of the lung surmounted by a dense air shadow, both encapsulated by fibrosis, is generally

recognized as the x-ray finding of a lung abscess. While admitting that the above description is a positive method of diagnosis, yet this represents but a single phase of the disease. Other phases have received too little attention. The first indication of a lung abscess is an increased density in the lung field. Without a significant history this is often called an area of infiltration, consolidation, or fibrosis and the real process overlooked, or the pus of an abscess may have the same density as the surrounding consolidation and escape detection for many days.[2] As the abscess matures, the changes seen on the x-ray films have more meaning. A sharper margin limits the process from the neighboring lung substance (Fig. 4). With the onset of suppuration an increased density of the periphery is noted. Liquefaction and rupture of the abscess follow. Pus is discharged into the bronchi and expectorated. Air fills the partially evacuated cavity, giving the fluid level, characteristic of a lung abscess (Fig. 5). Complete emptying of the liquid contents of a cavity is followed by a collapse of the walls. With healing there is a progressive shrinkage of the shadow until obliteration is complete. After a variable length of time the last traces of this process disappear (Fig. 6).

BRONCHOSCOPIC EXAMINATION

One-third of the patients in this study were given bronchoscopic examinations by the Ear, Nose and Throat Department, following a modification of the technic of Jackson.[3] A swelling of the affected bronchial mucous membrane or extensive granulation tissue was seen. In addition a thick plug of inspissated pus occluding the bronchus was observed in many patients, but the repeated aspiration of pus from the involved main bronchus assured the diagnosis.

DIAGNOSIS

Patients who complain of pain in the chest and expectoration a few weeks after an acute upper respiratory infection, a pneumonia, or an operation about the nose and mouth should be suspected of having a lung abscess. In early cases inconclusive physical signs and localized areas of infiltration as seen on roentgenograms are strongly presumptive evidence. Since the diagnosis is not always easy to make, all patients with a suspicious history deserve a careful physical examination, serial x-ray films, and a bronchoscopic examination.

DISCUSSION

Many causes have been responsible for lung abscesses. In an eleven-day-old infant a large solitary infected congenital cyst was found at postmortem. This was a rare cause. The question of aspiration of foreign bodies[4, 5] and infective emboli from tonsillectomies[6-11] has been fully discussed by others. Particularly significant was the work of Ochsner and Nesbit[12] who found that after a peritonsillar infiltration with procaine, iodized oil taken into the pharynx passed directly into the tracheobronchial tree.

Occasionally pus is aspirated from an upper lobe abscess into a lower bronchus. Heavy doses of codeine or opium so decrease the cough reflex that pus is allowed to gravitate into the lower bronchi without being expectorated. Faulkner[13] has called this "internal drainage," and he has shown that oil easily flows from one lobe of the lung to another, or even to the opposite lung after changes in posture. In some patients this process may be so slight that an early aspiration during an exploratory bronchoscopic examination will prevent secondary atelectasis of a lower lobe.

Infection of the lungs secondary to a process elsewhere is given as a common cause of lung abscess. While it is true that upper respiratory infections are common and lung abscesses rare, relationship between the two should not be considered casually. The high mortality (Table I) and the protracted disability of those that get well[14] should make us wary of this sequela of the "common cold." Pneumonias on the other hand are always considered serious, so that the patients are more carefully watched during the convalescent periods. An increase or a purulent sputum in the absence of signs of empyema, is quickly recognized as a lung abscess. Amebiasis was responsible for one case in this series, in which the hepatic abscess ruptured through the diaphragm. Secondary lung abscess after hepatic abscess perforations are often mentioned by the French.[15, 16] This explains the frequency with which they mention emetine as a curative drug for lung abscesses.

Obstruction to a sizeable bronchus as well as infection is necessary for abscess formation. Any infection of the parenchyma of the lung with free drainage would result in a capillary bronchitis. Phagocytosis of the invading organisms and swelling of the mucous membranes would be followed by the accumulation of excoriated mucous membranes and débris in the lumen of the bronchi and bronchioles. The bechic blast and the tussive squeeze would force this material[17, 18] into the larger bronchi where the cilial activity and the cough reflex would expel it. Van Allen[19] and others found that the absence of cough tended to give a more chronic lesion in the experimental animal. Thick pus, great swelling of the mucous membranes, or interference with the bechic blast and the tussive squeeze plug the bronchus. The trapped bacteria within the lung tissue multiply and go through the same phases as an abscess elsewhere. Inflammatory changes must extend to the pleura as pleural pain is so frequent and early a symptom. The digestive activity of the leucocytes softens the offending plug or thins it, so that it is no longer able to occlude the lumen of the bronchus. Erosion of the bronchial wall is a common mechanism, while rupture into the pleural cavity is an uncommon and serious complication.[20] Rarely, it may produce a bronchial fistula.

Drainage is then established. Progress from this point depends upon the character of the abscess walls. In thin-walled cavities evacuation of their contents results in the collapse of the walls. Healing takes place by fibrosis. Thick walls prevent this collapse and perpetuate this condition even though the cavity is empty.

Except for Jackson[18] and a few others[21, 22] most authors have considered lung abscess as a static or stationary clinical entity. Progressive changes are easily seen in serial x-ray films, and they seem to parallel those described occurring in tuberculous cavities.[23] Surgeons, in particular, are liable to make this error, probably because they operate upon so many chronic cavities. As soon as it was recognized that pain in the right lower quadrant of the abdomen with tenderness, rigidity, nausea, and vomiting meant an acute appendicitis, fewer cases were allowed to perforate and abscess formation was infrequent. When it is realized that dullness, râles, and suppressed breath sounds two or three weeks after an upper respiratory infection or pneumonia probably mean a lung abscess, an important step in the diagnosis of this condition will have been made, and fewer cavities will be allowed to develop thick rigid walls.

SUMMARY

1. An analysis of 101 cases of nontuberculous lung abscess is presented, whose ages ranged from eleven days to sixty-six years.

2. The following predisposing causes were given: upper respiratory infections, 30 per cent; pneumonia, 13 per cent; oral operations, 11 per cent; and other causes 16 per cent.

3. Solitary abscesses were found in every part of the lung, but they occurred most often in the right middle and lower thirds.

4. Three important early physical signs are: dullness, râles, and suppressed breath sounds.

5. Successive changes seen on serial x-ray films are discussed as an early means of diagnosis.

6. Bronchoscopic examination and aspiration should be done early in every case of lung abscess.

7. Earliest signs of the condition are stressed in an endeavor to promote more effective treatment.

636 Church Street, Evanston, Ill.

30 N. Michigan Avenue, Chicago, Ill.

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LABORATORY METHODS

A NEW, ONE-MINUTE METHOD FOR THE STAINING OF SPIROCHETES, SPIRILLA, SPERMATOZOA, AND RELATED ORGANISMS*

ARNOLD J. GELARIE, M.D., NEW YORK, N. Y.

IN THE experimental study of spirillosis and spirochetal diseases, it is often necessary that the blood of a large number of animals be examined at frequent intervals. Not only in such experimental studies, but also in the clinical examination of lesions of doubtful origin, is the physician called upon to employ some staining method as an aid toward identification. Of the present-day methods, dark-field examination is widely used. While this is highly satisfactory in the hands of an experienced technician, the general practitioner or casual laboratory worker frequently encounters difficulty. For this reason, some prefer to use Burri's India ink method. The latter is unsatisfactory, however, because of the following reasons:

1. Colloidal state of the ink,
2. Destruction of the colloidal state upon addition of salt solutions and resulting coagulation,
3. Presence of microorganisms and fungi,
4. Difficulty in making an even spread,
5. Breaks occurring in the smear simulating spirilla,
6. Difficulty in searching for single organisms.

It is obvious that with Burri's method, duplication of results is impossible. Other available methods such as Levaditi's silver impregnation or Giemsa's stain are cumbersome, complicated, time consuming, and require special technical knowledge.

The method herein described is the outcome of an effort to overcome the obstacles mentioned above. Numerous and exacting tests have proved it to be highly satisfactory; all conditions are sharply defined and duplication is possible at all times.

Directions for the preparation of the necessary solutions are as follows:

1. Fixing solution: Dissolve 2.5 gm. of recrystallized zirconyl chloride ($ZrOCl_2$) in 100 c.c. of 10 per cent sodium chloride solution.

2. Citric acid solution: Make a 10 per cent solution of recrystallized citric acid in distilled water.†

3. Staining solution: Dissolve 0.25 gm. of gentian violet in 100 c.c. of N/10 sodium lactate solution. The pH of this mixture should be approximately 8.4. If not, it should be so adjusted.

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†To avoid fungus formation, add a few crystals of thymol.

4. Solution of K_2HgI_4: Dissolve 0.5 gm. of mercuric iodide in 100 c.c. of 0.5 per cent potassium iodide solution.

5. Counterstain: Dissolve 0.2 gm. of methylene blue in 100 c.c. of distilled water and add 0.2 gm. of carbolic acid.*

The material to be examined is smeared on the slide in a thin layer and allowed to dry in the air (not over the flame). Cover with the zirconyl chloride solution, allow to stand five seconds and then wash with water. The zirconyl chloride solution acts as a fixative, replacing fixation over the flame. Then place a few drops of the 10 per cent citric acid solution on the smear, allow to remain for ten seconds and wash again with water. The slide is now ready for staining. A few drops of the staining solution are placed on the slide, allowed to remain for thirty seconds and then washed off with water. This is followed by momentarily

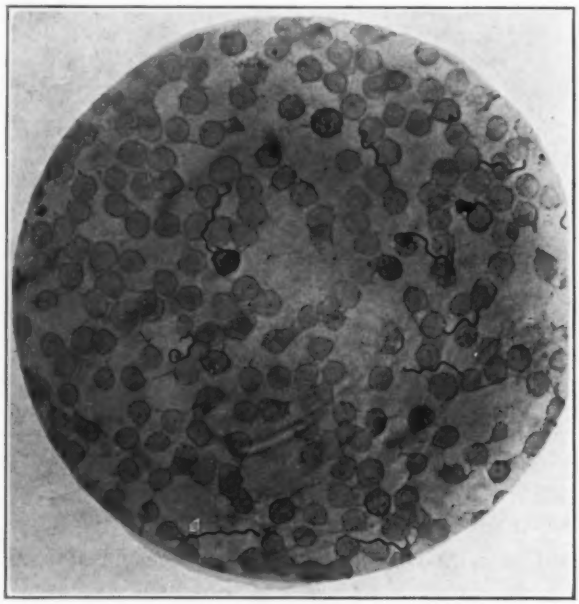

Fig. 1.—Spirillum recurrens from the blood of the mouse. Oil immersion, high power, magnification 1500.

covering the smear with a few drops of the complex mercuric iodide solution and again washing with water.† While a good stain is obtained at this point, uniformly excellent results may be obtained by counterstaining with aqueous carbol methylene blue solution for five seconds. The dye is then washed off and the slide dried with filter paper. The specimen is then examined by means of the oil immersion lens.

In staining spirilla, as from the mouse, one has to contend with the formed elements of the blood. In this case, their presence is advantageous since these cells offer a fine background against which the spirilla stand out very clearly. The cell outlines are sharply defined and the integrity of the cell body is

*For photographic purposes, use aqueous carbolfuchsin instead of methylene blue.

†A 0.01 per cent picric acid solution may be employed instead of the complex mercuric iodide solution. However, its use causes precipitation of the dye which obscures certain elements of the material under examination.

maintained. The red cells show a finely granular rose-colored cytoplasm, while the chromatin of the white cells stain blue. On the other hand, the spirilla are stained a deep purple,* differentiating them easily from surrounding tissue.

The *Treponema pallidum* obtained from the "Reizserum" differs from the above, in that formed elements are usually absent. The organism is stained a deep purple* and is very sharply defined in the clear, colorless field or in the faint, pink base formed by the stained coagulated serum.

With spermatozoa, to obtain exact detail, counterstaining is not recommended, due to overstaining of the organism. The different parts, head, neck, and tail, with their components, can be readily distinguished.

Figs. 1 to 4 show the result obtained with this method. It can also be used for staining other microorganisms such as gonococci, pneumococci, etc.

Fig. 2.—Spirochetes from the oral cavity.

The success of the above method can be accounted for by either chemical or physical theories of staining. From the results obtained, it would appear that there exist differences in the isoelectric points of the amphoteric material constituting the blood cells and that of the spirilla. As is well known, such ampholytic matter will "take" a basic dye if the hydrogen ion concentration of the staining solution is below its isoelectric point, and an acid dye if above that point. It is not difficult to imagine a difference in staining performance if to two such cellular elements the same dye be added. Should the amphoteric constituents of the spirilla possess a lower isoelectric point than those of the blood cells, the former will more strongly attract the basic dye present in the stain. Such appears to be the case.

*This is the color observed when artificial light is employed. However, when the source of light is natural, i.e. daylight, the organisms appear to be stained a deep blue, in contradistinction to the other elements present, which stain a light blue.

On the other hand, the simple principle of mordanting can also be applied here. The constituents of the smear may adsorb the added zirconyl chloride solution to different degrees. With subsequent addition of citric acid, the amounts of basic zirconium citrate formed within the blood cells and the or-

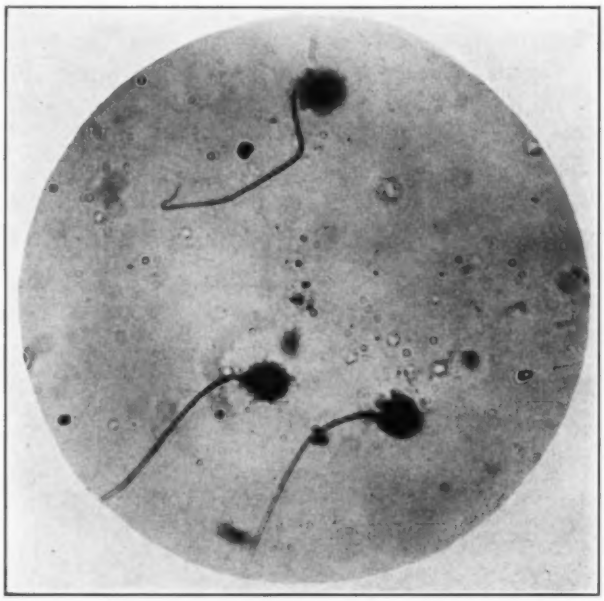

Fig. 3.—Human spermatozoa obtained from vaginal secretion.

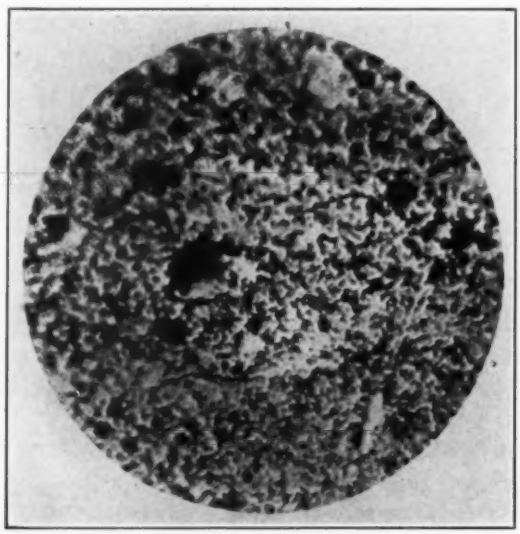

Fig. 4.—*Spirocheta pallida* from human chancre.

ganisms will be different. That such basic citrates are formed is easily demonstrated and their physical nature augurs well for the adsorption of staining materials. The constituent which more easily adsorbs the zirconyl chloride solution will contain more basic citrate and be more deeply stained.

Other salts such as the chlorides of aluminum, bismuth, cadmium, magnesium, mercury, thorium, uranium, copper, and tin were tried in the place of zirconyl chloride. While aluminum and thorium chlorides gave fair results, neither these nor the others were as satisfactory as the zirconium salt.

SUMMARY

1. A rapid method for staining spirilla, spirochetes, and spermatozoa is presented.

2. The organisms are found to stain a deep blue purple, easily identified from surrounding material.

3. Aluminum, bismuth, cadmium, magnesium, mercury, thorium, uranium, copper, and tin chlorides when individually substituted for zirconyl chloride in this method did not give as satisfactory a result.

4. The mechanism of the method is explained on a chemicophysical basis.

52 West Seventy-Fourth Street

THE INFLUENCE OF PHYSIOLOGIC SALINES IN COMPLEMENT FIXATION REACTIONS*

J. E. Faber, Jr., M.S., and L. A. Black, Ph.D., College Park, Md.

IN A SERIES of experiments preliminary to a standardization of methods for a study of various factors influencing complement, it was found that titrations of the same hemolysin varied greatly. Various suggestions have been made in the literature regarding the possible effects of saline, and since this seemed to be the cause of the present variations in hemolysin titration, a series of experiments were conducted on salines prepared in various ways.

In a study of the precise titration of complement, Brooks[1] recommended a solution similar to Ringer-Tyrode's solution containing 8 gm. of sodium chloride, 0.2 gm. of potassium chloride, 0.2 gm. of calcium chloride, 1 gm. of sodium bicarbonate, and 0.05 gm. of monobasic sodium phosphate, made up in a liter of distilled water. This physiologically balanced solution was used in place of physiologic saline as a diluent.

The consideration of hydrogen ion concentration seemed very important to Mason and Sanford[2] and they urged the use in serology of a constant physiologic salt solution. Their buffered solution was prepared by adding 7 gm. of sodium chloride, 1.7 gm. of secondary sodium phosphate, and 0.2 gm. of primary potassium phosphate to each liter of double distilled water. This gave a solution with a pH of 7.4 to 7.8. They stated that the optimum hydrogen ion concentration for amboceptor titration was between pH 7.0 to 8.4. Boiling and the addition of carbon dioxide changed the concentration of hydrogen ions in distilled water too easily, while tap water did not change as greatly because of the natural buffers present in such water.

*From the Department of Bacteriology, University of Maryland, College Park.
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The work of Rockwood[3] indicated that the effective range of acidity for biologic hemolysis was between the pH of 6.0 to 8.3 with a broad optimum near neutrality. Acid would partly destroy the amboceptor and complement. Mason and Sanford's buffered salt solution, although stabilizing a variable factor in the Wassermann test, did not buffer the final mixture to neutrality since except in extreme cases the buffering value of the cells is enough to accomplish this.

Kellogg and Wells[4] did not consider the reaction of saline solutions of any great importance. Salines prepared with tap and distilled water of the same pH gave a low titration with distilled water and a very high one with tap water, which they considered was due to the presence of magnesium in the tap water. By adding 0.1 gm. of magnesium chloride or sulphate to a liter of physiologic saline solution the specific lysis of red cells was increased giving higher complement and amboceptor titers.

Kolmer[5] states that sterilization of saline in an Arnold for an hour (for 1,000 c.c. or a shorter time for a smaller bulk) is preferred to autoclaving because of the concentration of salt in the latter due to the additional loss of water. Kolmer[6] also recommends the use of 8.5 gm. of chemically pure sodium chloride as the optimum amount of salt. He mentions that the pH of the saline is of practical importance but not the sole factor in selecting a good saline. According to Kolmer the buffered solution of Mason and Sanford is somewhat hemolytic when made up in distilled water and is not as satisfactory as when prepared with Philadelphia tap water, although the range of pH for both was 7.3 to 7.4. He is of the opinion that "the natural buffers of tap water are likely to be more satisfactory in relation to serum hemolysis than artificially buffered distilled water." If saline prepared of distilled or tap water should be unsatisfactory Kolmer recommended that 0.1 gm. of magnesium sulphate or chloride be added to each liter in accordance with Kellogg and Wells.[4]

Recent books on laboratory methods usually recommend distilled water for physiologic saline. Wadsworth[7] suggests that physiologic salt solution containing 0.85 per cent sodium chloride be used for preparing suspensions of red cells, and for making all dilutions. The required quantity of distilled water is added to the salt, dissolved, filtered through paper, dispensed and sterilized at 121° C. for twenty or thirty minutes, depending on the bulk. It was unnecessary to compensate for small losses of water during sterilization, which usually resulted in a salt solution of about 0.88 per cent. According to Kolmer and Boerner,[8] one liter of tap or distilled water should be added to 8.5 gm. of dry chemically pure sodium chloride, filtered through paper into a flask fitted with a gauze-covered cotton stopper and sterilized in an Arnold for an hour.

Gradwohl[9] recommends the use of 8.5 gm. of sodium chloride per 1,000 c.c. of distilled water. This solution is filtered through paper and 500 c.c. brown bottles are filled about two-thirds full, plugged with nonabsorbent cotton, which is then covered with paper and the bottles are then sterilized in the autoclave for thirty minutes at 15 pounds' pressure. He states that fresh saline should be made once or twice a week, and if not stored in brown bottles, should be placed in a dark cabinet.

In the standard procedure of the A. P. H. A. for the complement fixation test[10] it is only stated that "the diluent for all reagents should be a salt solution containing chemically pure components isotonic for red cells."

EXPERIMENTAL

Hemolysin Titration.—The hemolysin used in all of these experiments was of the same lot prepared by the Difco Laboratories and stated to have a titer of 1:5,000 when used with 0.5 c.c. of 2 per cent corpuscles and 0.3 c.c. of 1:30 complement. Complement was secured by pooling the blood of healthy guinea pigs whose titer was already known to be satisfactory, allowing this to stand at room temperature for one hour, refrigerated for twenty-four hours and centrifuged if necessary. The complement was used the same day (except in the last complement experiment when it was used the second day). The corpuscles were obtained from a ewe, defibrinated with glass beads, washed three times or more for ten minutes each at 2,200 r.p.m., packed by centrifuging for twenty minutes, made up to 50 per cent suspension, refrigerated, and used for not longer than two days.

Saline solutions were prepared with 0.85 per cent sodium chloride made up in various waters, and of these some were used unheated, some were placed in flowing steam for thirty minutes, and others autoclaved for thirty minutes. Determinations were made of the pH of these solutions just after use, using a quinhydrone pH indicator at first and later a colorimetric method, using standards and solutions that had recently been checked.

A modified Ringer's solution was used in the first two experiments, consisting of 8.0 gm. NaCl, 0.14 gm. KCl, 0.12 gm. $CaCl_2$ and 0.20 gm. $NaHCO_3$. The various salines prepared were used in each case for dilution of the 1:100 hemolysin and for bringing up the volume. A tap water saline which had been steamed was used for making the first 1:100 dilution of hemolysin and also for diluting the complement and corpuscles.

In the second two experiments a phosphate buffered saline like Mason and Sanford's[2] was used, except that only single distilled water was used. Another tap water was secured from Washington, D. C., since their tap water is substituted generally there for distilled water for use in batteries, and is of somewhat different character than other tap water available. The various salines prepared were used in each case for the entire dilution of glycerinated hemolysin and for bringing up the volume. An autoclaved tap water was used in these last two experiments for diluting the complement and corpuscles. In Experiment IV the saline solutions used were those used the preceding day in Experiment III.

In Protocol 1 the arrangement used for each titration of hemolysin with each saline is shown (except additional intermediate dilutions of 1,500-5,500 which were made in the last experiment). The results obtained are tabulated in Table I.

The results of the first experiment in *hemolysin titration* showed a decided increase in titer by using saline prepared from tap water (1:3 or 4,000) compared with saline with distilled water (1:1,000). An increased titer was also

indicated in the salines that had been placed in flowing steam for thirty minutes compared with those unheated. The pH of the solutions also varied with the titer with the unbuffered salines, whereas with a modified Ringer's solution there was no correlation of pH and hemolysin titer. Ringer's solution steamed was of equal titer as tap water steamed but unheated Ringer's solution gave a very low titer corresponding to that with distilled water.

Repetition of this experiment after two days with freshly prepared salines gave comparable results as shown by Experiment II. In this case the titer with tap water was not as high as with Ringer's solution (steamed) and likewise the pH of the tap water was not as high as in the preceding experiment.

Other experiments were then conducted on complement titrations but after a month, Experiments III and IV were performed, discontinuing Ringer's solution and adding a phosphate buffered solution (Mason and Sanford's) and another tap water, and including an autoclaved saline in each experiment. Distilled water saline was again lowest in titer, distilled water buffered with phos-

PROTOCOL 1. TITRATION OF HEMOLYSIN

TUBE NO.	A	B	C	D	E	F	G
Diluted hemolysin c.c.	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Complement (1:10) c.c.	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Corpuscles (2%) c.c.	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Saline c.c.	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Incubate at 37° C. for One Hour							
RESULTS							
Hemolysin Dilution 1	1,000	2,000	3,000	4,000	5,000	6,000	8,000

TABLE I

HEMOLYSIN TITRATION WITH VARIOUS SALINES

SALINE*		I TITER 1 :	I pH	II TITER 1 :	II pH	III TITER 1 :	III pH†	IV TITER 1 :	IV pH†
Distilled									
	DU	1000	5.6	1000–	5.9	2000	6.2	1500	6.4
	DS	1000+	6.1	1000+	6.0	2000	6.3	2000	6.4
	DA					2000+	6.3	2000	6.4
Tap									
	TU	3000	7.1	2000–	7.0	3000	7.4	2500	7.4
	TS	4000+	7.3	2000+	7.0	3000	7.4	3000	7.4
	TA					4000+	7.6	4000	7.4
Tap Ringer's									
	TRU	1000	7.9	1000–	7.9				
	TRS	4000	7.8	4000	7.8				
Distilled phosphates									
	DPU					3000	7.1	3000	7.1
	DPS					3000	7.3	3000	7.3
	DPA					3000	7.3	3500	7.3
Tap Washington									
	TWU					4000+	7.8	4500	8.0
	TWS					4000+	8.0	5000	8.0
	TWA					4000+	8.2	5000	8.2

*Complement and corpuscles were diluted with tap water saline, steamed in I, II and autoclaved in III, IV.

D, distilled
T, tap
R, Ringer's
P, phosphates
W, Washington
†Colorimetric pH.

U, unheated
S, steam (flowing)
A, autoclaved
– not quite complete
+nearly complete in next higher dilution

phates considerably higher in titer and but little below the tap water, and highest of all was the other tap water used now for the first time. Experiment IV was performed with the same reagents used the preceding day in Experiment III but with a series of intermediate dilutions to allow a more accurate record of titer. A higher titer was usually obtained with autoclaved salines than with unheated salines, the salines heated in flowing steam sometimes being of the same titer as autoclaved salines and sometimes lower, and sometimes as low as the unheated salines. In all cases the pH of the same salines heated or unheated were quite close and there was again a correlation of pH with the titers obtained.

COMPLEMENT TITRATION

Since the results had indicated the influence of the water used in preparing saline upon hemolysin titration, a series of experiments were planned to check these effects upon complement titration.

The reagents used were prepared as indicated under hemolysin titration. A solution similar to Brooks'[1] Ringer-Tyrode's solution was also prepared. This formed such a precipitate with tap or distilled water that the solution was unfit for use because it obscured the results.

In Protocol 2 the arrangement used for each titration of complement for each saline is shown. In the experiments themselves the various salines were used for only *one reagent* in each test, the other reagents in each case being prepared with tap water saline heated in flowing steam for 30 minutes, which was also used for preparing the 1:100 dilution of hemolysin. The results obtained are tabulated in Table II.

A further series of experiments were conducted in which the varying salines were used for *each reagent* in the test, no other saline being used in any place with the exception of the preliminary washing of the blood corpuscles and their preparation to a 50 per cent suspension. In addition, the effect of the addition of magnesium chloride[4] was tested with one distilled and one tap water. These results are presented in Table III.

In the three trials of *complement titration* in which the *volume* alone was made up with varying salines (the amount varying from 1.7 to 1.9 c.c.), distilled water was poorer than tap in one, about the same in one, and better in one trial. The addition of phosphates to distilled water improved the titer slightly under these conditions. The improvement in titer due to heating was also less striking in these experiments, the heated being better in four, the same in three and in one case the autoclaved was slightly poorer than the unheated.

When the *corpuscles* alone were diluted with the various salines (0.5 c.c. being used in each tube), the distilled water gave about the same titer as tap water and the addition of phosphates improved the titer in one series and did not in the other series used. However, there was a noticeable uniform increase in titer of steamed over unheated salines and of autoclaved over steamed salines. It would seem that the increased evaporation of water and accompanying concentration of salts due to heating the saline did affect the red cells to such an extent that the complement titer was correspondingly improved.

The influence of the varying salines used for dilution of *hemolysin* alone (0.5 c.c. being added) further than 1:100 did not seem to be evident. There was little difference between distilled and tap water salines and except in one case, with phosphate buffered water. Likewise there was no apparent effect of heated compared with unheated waters.

PROTOCOL 2

TITRATION OF COMPLEMENT

TUBE NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Complement 1:10 c.c.	0.06	0.08	0.10	0.12	0.14	0.16	0.18	0.20	0.22	0.24	0.26	0.28	0.3	0.3	0	0
Hemolysin 2 units c.c.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0	0.5	0
Sheep cells 2% c.c.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Saline c.c.	1.94	1.92	1.9	1.88	1.86	1.84	1.82	1.8	1.78	1.76	1.74	1.72	1.7	2.2	2.0	2.5

Incubated at 37° C. for one hour.

TABLE II

COMPLEMENT TITRATION WITH VARIOUS SALINES. RESULTS EXPRESSED IN C.C. OF 1:10 COMPLEMENT REQUIRED

SALINE*	VOLUME (1.7-1.9 C.C)	CORPUSCLES (0.5 C.C.)	HEMOLYSIN* (0.5 C.C.)	COMPLEMENT (0.09-0.18 C.C.)	pH
Distilled					
DU	0.18	0.16	0.12	0.14	5.5
DS	0.18	0.14	0.14	0.14	5.8
DA	0.18	0.10	0.14	0.14	5.8
Tap					
TU	0.14	0.18	0.18	0.14	6.7
TS	0.12	0.14	0.18	0.14	6.9
TA	0.10	0.12	0.18	0.14	7.1
Distilled					
DU	0.10	0.18	0.12	0.08	5.5
DS	0.10	0.14	0.14	0.08	5.6
DA	0.10	0.12	0.14	0.08	5.9
Tap					
TU	0.14	0.16	0.14	0.08	6.4
TS	0.12	0.14	0.14	0.08	6.6
TA	0.12	0.12	0.14	0.08	6.8
Distilled phosphates					
DPU	0.10	0.12	0.10	0.10	6.6
DPS	0.08	0.10	0.12	0.10	6.9
DPA	0.08	0.10	0.10	0.10	6.9
Distilled					
DU	0.16	0.22	0.18	0.18	6.0
DS	0.16	0.20	0.18	0.18	6.0
DA	0.16	0.18	0.18	0.18	6.3
Tap					
TU	0.18	0.20	0.18	0.18	6.8
TS	0.18	0.18	0.18	0.18	7.1
TA	0.16	0.16	0.18	0.16	7.1
Distilled phosphates					
DPU	0.14	0.22	0.18	0.20	6.7
DPS	0.14	0.22	0.18	0.18	6.9
DPA	0.16	0.18	0.20	0.18	6.9

*A tap water saline (steamed) was used in preparing all other reagents except the one being tested (including the 1:100 hemolysin).

D, distilled
T, tap
P, phosphates
U, unheated
S, steam (flowing)
A, autoclaved

The effect of varying the saline in the dilution of the *complement* alone was striking in the almost completely identical titers regardless of the salines or of their exposure to heat. Only 0.09 to 0.18 c.c. of saline would have been used in any test and in a volume of 3 c.c. containing a suitable saline such as heated tap water (in these experiments), the influence of the one variable added in such a small amount was negligible. This may account for the similar results obtained with hemolysin, for although 0.5 c.c. was used, the first 1:100 dilution was made with a steamed tap water saline.

TABLE III

COMPLEMENT TITRATION WITH VARIOUS SALINES

SALINE*		TITER (C.C. OF 1:10)	pH†
Distilled			
	DU	16	6.6
	DS	20	6.4
	DA	18	6.6
Tap			
	TU	14	7.6
	TS	14	7.6
	TA	16	7.8
Distilled phosphates			
	DPU	08	7.3
	DPS	10	7.3
	DPA	10	7.3
Distilled magnesium			
	DMU	08	6.6
	DMS	08	6.5
	DMA	08	6.7
Tap magnesium			
	TMU	10	7.5
	TMS	12	7.5
	TMA	10	7.8

*Diluent same for each reagent and for bringing up volume.
D, distilled
T, tap
U, unheated
S, steam (flowing)
A, autoclaved
P, phosphates
M, magnesium chloride
†Colorimetric pH.

The actual titers obtained in the three series varies, of course, due to different complement being used. There was apparently no correlation of titer of complement and pH, unless in the cases where there was an effect due to heating, as here the pH also was slightly higher in the heated salines.

When the same saline was used as the *only diluent* for a complement titration, saline made with our distilled water required a larger amount of complement than did that made with our tap water. The addition of phosphates to the distilled water reduced by about half the amount of complement needed in this experiment. The addition of magnesium chloride to our distilled water likewise reduced the amount of complement needed to less than half and with our tap water reduced the amount of complement needed, although not to as small an amount as with distilled water.

The difference between distilled and tap water was not as great again in the last experiments as with hemolysin titration. It should be noted, however,

that there was less difference between the pH of the two types of saline, and that of the distilled water saline was near neutrality instead of being acid as usual.

Again with the buffered solutions there was no correlation between the heated or unheated salines. There was little difference in the last experiment between heated and unheated solutions of distilled or tap water, but it should be noted that the pH of these solutions were near neutrality and there was not the increase in pH with the increase in heating usually found in preceding experiments. The pH of unheated solutions was as high or in some cases higher than the heated, which may account for the small discrepancies in titer of the unheated over the heated salines.

DISCUSSION

Mason and Sanford[2] stated that the optimum pH for amboceptor titration was 7.0 to 8.4. The highest hemolysin titer in our experiments was obtained with a tap water saline at the highest pH reached, 8.2. Low titers were secured with a Ringer's solution unheated at a pH of 7.9 but with this exception there did seem to be some correlation between pH of the saline and hemolysin titration, a satisfactory titration not being obtained unless the pH was above 7.0. Two tap waters gave higher titers however than did the phosphate buffered saline of Mason and Sanford.

The addition of magnesium salts to a distilled water saline after Kellogg and Wells[4] gave the highest complement titer secured. In this case the pH of these two salines remained the same, although the titer was doubled.

Although a steamed saline as recommended by Kolmer[5] gave good results, a higher hemolysin titer was secured with our tap water by an autoclaved saline.

Freshly prepared solutions were used in all of our experiments except the last hemolysin titration when the saline was used a second day. The results were equally as good, and it is believed that a tap water or buffered saline could be kept for some time without changing materially.

In view of the usual recommendations that saline be prepared with sodium chloride and distilled water, it is suggested that attention could well be given to the local waters available and possibly some uniform saline adopted for diluent in any standard proposed for complement fixation reactions.

SUMMARY AND CONCLUSIONS

1. In hemolysin titration saline prepared from tap water gave a decided increase in titer over that prepared from distilled water. In complement titration there was not such a difference in titer.

2. Hemolysin titrations were increased by heating saline in flowing steam for thirty minutes compared with unheated salines and in some cases there was a further increased titer of autoclaved over steamed salines. In complement titrations where the salines were nearer neutrality there was little difference in titer between heated and unheated salines.

3. In hemolysin titrations the pH of the unbuffered salines increased with the increase in titer, but there was not such a correlation in our complement titrations where the salines were nearer neutrality.

4. A Ringer's solution when heated gave a satisfactory hemolysin titer equal to that of tap water, but if unheated it had a very low titer.

5. A phosphate buffered distilled water saline gave a satisfactory hemolysin titer comparable to that of tap water, although the two tap waters used gave higher titers.

6. While a steamed saline gave good results, autoclaving of our tap water gave a higher titer and made it satisfactory for hemolysin titration even though the titer did not reach that of another tap water especially suitable which gave the highest hemolysin titer obtained.

7. The highest hemolysin titer was obtained at the highest pH of 8.2 and a satisfactory titer of unbuffered saline was not obtained unless above pH 7.

8. A modified Ringer-Tyrode's solution that has been suggested for complement titration gave such a precipitate that it could not be used.

9. In complement titrations where a satisfactory saline was used for diluting all reagents but one, the use of an inferior or superior saline either for making up the volume or for diluting hemolysin or complement alone did not result in any uniform difference between distilled or tap water salines or between heated or unheated salines.

10. When the corpuscles alone were diluted with the various salines there was a noticeable uniform increase of titer of steamed over unheated saline and of autoclaved over steamed salines, indicating that the increased evaporation and concentration of salts did affect the titer.

11. When one saline was used throughout complement titration as the only diluent, saline made with our distilled water required more complement than when tap water was used.

12. The addition of phosphates to our distilled water saline reduced the amount of complement needed about one-half.

13. The addition of magnesium chloride to our distilled water saline reduced the amount of complement needed to less than half and gave the highest complement titer obtained. Our tap water saline was also improved by the addition of magnesium chloride.

14. It is believed that a satisfactory tap water or buffered saline can be kept for some time without changing materially.

15. The results suggest that in order to secure the highest titer a determination should be made of the best water available locally for the preparation of saline for serologic tests.

16. The results indicate the desirability of a uniform saline in any proposed standard procedure for complement fixation reactions.

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A METHOD OF QUANTITATIVE AND QUALITATIVE ESTIMATION OF PLATELETS IN THEIR OWN PLASMA*

ELEANOR I. LESLIE, M.D., AND HEYWORTH N. SANFORD, M.D., CHICAGO, ILL.

THE rapidity with which tissue fluid and glass cause disintegration of platelets is well known. In puncture wounds it is impossible to avoid the admixture of some tissue fluid and even in blood taken by venipuncture, this may occur. Fonio,[1] Nygaard,[2] Cummings,[3] and Lampert[4] have used various fluids and attempted to avoid contact with glass. These methods lessen such errors but offer no means of checking to what extent the platelets may have been broken down before the count is made. The method here described avoids the destructive action of both tissue fluid and glass and affords a means of estimating the quality as well as the number of platelets.

METHOD

The procedure is as follows: no anticoagulant is used as such substances mask the extent to which platelets may be broken down in the original blood sample and so interfere with correct judgment of results. The blood is taken from a vein or the longitudinal sinus into an iced, oiled syringe and introduced after the removal of the needle into paraffined tubes imbedded in ice. The tubes used were 10 by 100 mm. and were paraffined with a mixture of about two-thirds paraffin and one-third mineral oil; the addition of the mineral oil is necessary to prevent cracking of the paraffin when the tube is chilled to 0° C. The tubes are then packed in ice in a large centrifuge cup and centrifuged for one and one-half minutes at 5,000 r.p.m., followed by a period of five minutes at the same speed.

The platelet count is made after the one and one-half minutes centrifuging as follows: a few grains of heparin are placed on a paraffined watch crystal resting on ice and to these are added several drops of the plasma. Following thorough mixing, the plasma is diluted 1-10 in the ordinary pipette used for counting white blood cells. The pipette must be thoroughly chilled and iced normal saline is used as the diluent. Shaking for one minute suffices and after discarding the first three drops, the mixture is introduced into a chilled counting

*From the Department of Pediatrics, Rush Medical College and the Presbyterian Hospital.
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chamber. With the base of the counting chamber resting in iced water, fifteen minutes is allowed for settling and the count is made under high power. The platelets in eighty small squares are counted and since this represents one-fiftieth of a cubic millimeter and the dilution is 1-10, the count is multiplied by 500 to obtain the number of platelets in a cubic millimeter of plasma. In cases of thrombocytopenia, no dilution is necessary and after mixture with the heparin the plasma may be run directly into the chilled counting chamber. Counting eighty small squares, the result is multiplied by fifty to obtain the number of platelets per cubic millimeter. Heparin inhibits the breaking down of platelets by glass provided a low temperature is maintained. Other diluents such as sodium citrate or 14 per cent magnesium sulphate might be used instead of the heparin and saline, but when these are used in a 1-10 dilution the platelets showed a tendency to clump. Kristensen's fluid is satisfactory when fresh, but it deteriorates rapidly. The platelet counts obtained by the method described must be considered plasma platelet counts and hematocrit readings are necessary in order to interpret them in terms of counts for the whole blood. Inasmuch as the platelets rise with the plasma and are evenly distributed, the following proportion holds: the platelet count for the whole blood is to the plasma platelet count as the plasma volume is to the blood volume; in other words, the plasma platelet count multiplied by the plasma percentage gives the platelet count for the whole blood.

The plasma is now transferred to two 8 by 75 mm. paraffined tubes. One tube containing about 0.5 c.c. (15 drops) taken from the center of the plasma layer is set aside in ice for a determination of the coagulation time of the whole platelet containing plasma, designated as plasma A. The other containing 1.0 to 2.0 c.c. of plasma is suspended in a Pyrex bottle filled with ice which fits in the large centrifuge cup. The plasma is then centrifuged for one and one-half hours at 10,000 r.p.m. With an ordinary room temperature it is necessary to repack with ice every half hour. During this long centrifuging, all intact platelets are thrown to the bottom of the tube where they may be seen as a grayish mass. The supernatant plasma is platelet free but may contain products of platelet disintegration. Platelet-free plasma is designated as plasma B. An amount of plasma B equal to the amount of plasma A is transferred to a similar paraffined tube for determination of coagulation time. Likewise an equal amount of plasma is left in the bottom of the tube containing the platelets which have been thrown down by the long centrifuging for determination of the coagulation time of a platelet-rich plasma, plasma C. For determination of coagulation times, the tubes containing plasma A, B, and C are transferred from ice to a water-bath kept at 25° C. When a solid clot is formed the fibrin is removed by twirling a wooden applicator stick in the clot, squeezing out all the remaining fluid. If a clot re-forms, the fibrin is again removed. In platelet-free plasma, clotting continued to occur throughout a long period, often thirty minutes. In a platelet-rich plasma, it is usually complete in four to six minutes. In the tables the two figures represent the beginning and the completion of coagulation.

With this procedure we have for consideration a plasma platelet count, the coagulation time of natural plasma with its full number of platelets, the coagu-

lation time of platelet-free plasma and the coagulation time of platelet-rich plasma. If any difficulty is encountered in getting the blood so that there is the slightest addition of tissue fluid or cerebrospinal fluid, the results are unreliable. This can usually be detected when the platelet count is made. It will be lower than anticipated and the platelets show clumping and variation in size. A coagulation time for plasma A of under ten minutes is also strong evidence that there has been faulty technic. If the procedure is carried through in these instances to the long centrifuging, the plasma usually coagulates during the time of centrifuging or rapidly after removal from ice to the water-bath. In all studies of coagulation factors based upon timing, one should know whether or not there has been addition of tissue fluid to the blood. This method of checking is far from perfect but when anticoagulants are used, no check at all is possible.

RESULTS

Normal adults show a plasma platelet count varying between 250,000 and 500,000. The coagulation time for plasma A ranges between ten and thirty minutes, for plasma B from twenty-five to one hundred minutes, and for plasma C from five to fifteen minutes.

The first deviation from these standards is shown in cases with hemorrhagic symptoms, but with normal or high platelet counts (see Table I). Since these

TABLE I

REMARKS	PLASMA PLATELET COUNT	COAGULATION TIME IN MINUTES		
		PLASMA A	PLASMA B	PLASMA C
Normal	250,000-500,000	10-30	25-100	5-15
Mr. R., petechiae, fever; died with ulcerative colitis (rapid sedimentation)	825,000	22-25	120-138	8-11
M. McH., boy, 8 years following period of bleeding from intestine	550,000	32-34	240-360	17-21
J. McE., boy, 7 years, petechiae, endocarditis	470,000	20-25	115-140	15-17

cases all had normal or high platelet counts, the delayed coagulation would seem necessarily to be due to an increased platelet resistance. No studies have as yet been made on patients with hemophilia.

Cases of thrombocytopenia divide themselves according to this method of study into two distinct groups. The first shows prolonged coagulation times for all plasmas which is sometimes very great for the platelet-free plasma (see Table II). The second group shows a wide variation in the coagulation time of plasma A, but characteristically yields a platelet-free plasma which has a coagulation time of about the same length or even less than that of the whole plasma (see Table III).

In the first group we have two cases of acute leucemia in children. Platelet counts taken without centrifuging the blood, because of the rapid development of a plasma layer, were in the neighborhood of 30,000 for one and 107,750 for the other. Plasma A coagulated in thirty-eight to forty-five minutes, plasma B in from seventy to 180 minutes, and plasma C in from twelve to twenty-five

minutes. A third case, E. C., aged six years, showing a single episode of purpura with no recurrence as far as is known, had a platelet count of 46,000, a coagulation time for plasma A of twenty minutes, for plasma B of 430 minutes, and for plasma C of fifteen minutes. The subsequent platelet counts have been 400,000, 475,000, and 550,000 and the coagulation times for all plasma within the normal range. A fifth case, Shirley, showing marked purpuric manifestations of several weeks' duration, had a platelet count of 25,000, a coagulation time for plasma A of forty minutes, for plasma B of 180 minutes, and for plasma C of twenty min-

TABLE II

REMARKS	PLASMA PLATELET COUNT	COAGULATION TIME IN MINUTES		
		PLASMA A	PLASMA B	PLASMA C
L. A., boy, leucemia, rapid sedimentation time, hematocrit 15 per cent	34,500	30-45	55-70	20-25
Day following transfusion	23,500	30-45	45-100	15-35
E. F., boy, leucemia, rapid sedimentation time	107,750	30-38	138-	10-12
Lois, colored girl, single purpuric episode, rapid sedimentation time, hematocrit 20 per cent	23,500	38-50	90-200	12-20
E. C., boy, single purpuric episode	46,000	19-21	370-430	13-15
Question of recurrence, few bruises 1 month later	400,000	14-17	35-45	9-
3 days later, recovered	475,000	10-	22-30	5-
14 months later, no recurrences	550,000	13-18	50-60	8-10
Shirley, girl, purpuric symptoms 2-3 weeks' duration. Died pulmonary hemorrhage. Question aleucemic leucemia	25,000	30-40	180-	20-

TABLE III

REMARKS	PLASMA PLATELET COUNT	COAGULATION TIME IN MINUTES		
		PLASMA A	PLASMA B	PLASMA C
H. B., girl, 8 years, numerous petechiae, long bleeding time	10,000	180-	120-	21-31
E. W., boy, 13 years, repeated bleeding and petechiae many years	1,250	60-	45-	15-
1 day later	2,250	40-50	30-35	15-
Day following transfusion	4,000	22-31	17-23	6-11

utes. The child died of pulmonary hemorrhage. Histologic study of the bone marrow showed degeneration of the megakaryocytes with a leucocytic reaction raising the question of an aleucemic leucemia.

In the second group we have a girl, H.B., aged eight years, who has shown petechiae and hemorrhages of the mucous membranes over a long period of time. Bleeding of the gums continued for four days following the extraction of decayed teeth. Her platelet count was 10,000, the coagulation time for plasma A 180 minutes, for plasma B 120 minutes, and for plasma C twenty-seven minutes. A

boy, E. W., aged thirteen years, has had numerous episodes of hemorrhage into the skin and from the mucous membranes. On admission, his platelet count was 1,250. Plasma A required sixty minutes for coagulation, plasma B forty-five minutes, and plasma C fifteen minutes. The next day the platelets were 2,250 and the coagulation time for plasma A was fifty minutes, for plasma B thirty-five minutes, and for plasma C fifteen minutes. A transfusion was given on the day following admission and the following day the platelets were 4,000, the coagulation time for plasma A thirty-one minutes, for plasma B twenty-three minutes, and for plasma C eleven minutes.

The findings in these cases of thrombocytopenia suggest that in the first group we are dealing with platelets which, though reduced in number, are fairly normal in character. They undergo relatively little disintegration during the process of centrifuging them out of the plasma and the coagulation time of plasma A is prolonged in most cases, proportionately to the reduction in total platelet count. There is the possibility that the prolongation of coagulation represents an increased resistance as well as a reduction in numbers. This is particularly indicated in several of the cases by the prolonged coagulation time of the platelet-rich plasma. In the second group, where we are dealing with cases of long-standing purpura, resembling the syndrome described by Werlhof, the platelets do not show a normal resistance. They are broken down during the long centrifuging, yielding a platelet-free plasma which coagulates more rapidly than plasma A. Whether this is due to properties inherent in the platelets or to changes produced during the course of their circulation in the blood stream must be the subject of further investigation. It is possible in these cases that the platelets are produced by the bone marrow in normal numbers but that many undergo destruction in the blood stream and that those remaining show a tendency to increased fragility.

CONCLUSIONS

1. A method of estimating platelets quantitatively and qualitatively in their own plasma is described.

2. It depends upon a consideration of the coagulation times of whole plasma, platelet-free plasma and platelet-rich plasma.

3. By use of chilled, oiled syringes and paraffined tubes, errors due to contact with glass are avoided, and as no anticoagulant is used, it is possible to check fairly accurately whether or not the blood has been contaminated with tissue fluid. If this has occurred, the blood is unsuitable for further study.

4. Among miscellaneous cases with normal or high platelet counts which had presented hemorrhagic symptoms, three were found which had unusually resistant platelets.

5. Cases with thrombocytopenia divided themselves into two groups, one with platelets showing normal or increased resistance, and the other with platelets giving evidence of increased fragility. From these observations, cases in the first group belong to the symptomatic type of purpura and those in the second group to the so-called essential thrombocytopenia type.

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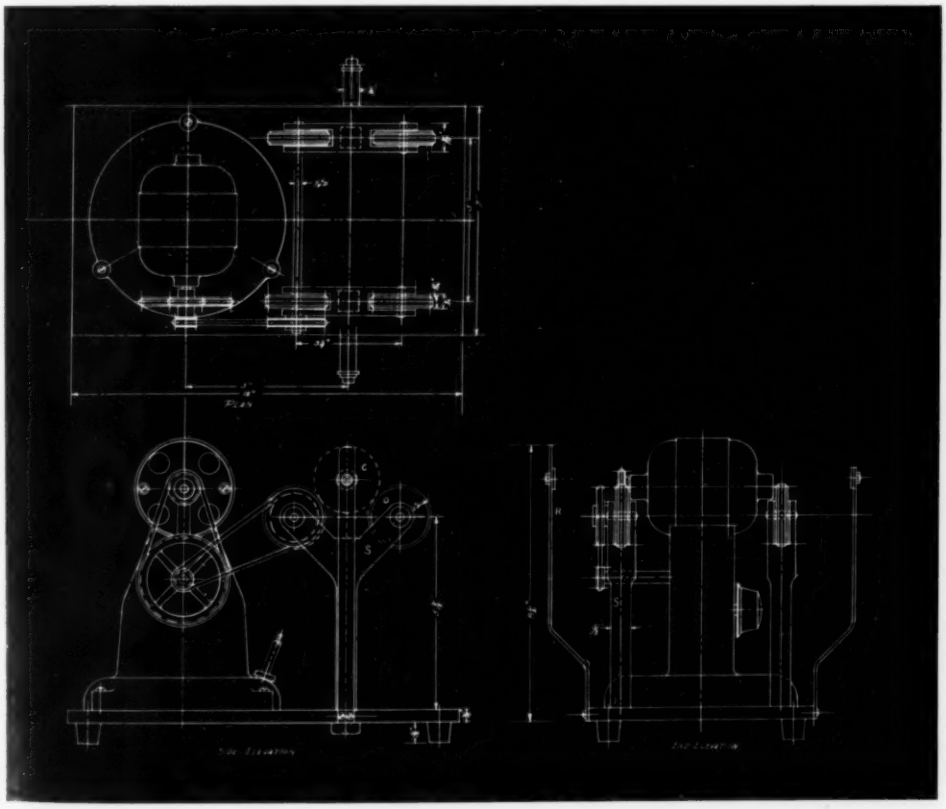
636 CHURCH STREET, EVANSTON
952 N. MICHIGAN AVENUE, CHICAGO

A BALL MILL FOR GRINDING SMALL QUANTITIES OF BACTERIA*

JOHN F. NORTON, JOHN H. DINGLE, AND T. HERBERT SHENSTONE,
KALAMAZOO, MICH.

IN THE course of studies on the antigenic constituents of bacteria, it was necessary to employ a ball mill which would conveniently grind small amounts of bacteria in either wet or dry condition. Such a mill is described here.



Pyrex glass or stainless steel containers, 8 inches long and 1¾ to 3 inches in diameter, are employed. These may be closed with rubber stoppers or

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fitted lids. Approximately 1,000 stainless steel balls,* ⅛ in. (3.2 mm.) in diameter, are placed in the container with the bacteria. Both container and steel balls are easily sterilized if asepsis is desired. The container (*C*) is placed upon two sets of rubber rollers (*O*), supported by V-shaped standards (*S*), and is held in place by adjustable retainers (*R*) at each end as shown in the accompanying drawing. Base, standards, and retainers are constructed of cold rolled steel. Rollers are of rubber with brass side plates. Power is supplied by a variable speed motor,† and speed is controlled by the motor or by pulleys to obtain a speed of 140 to 250 revolutions per minute. The motor and rollers are connected by heavy rubber belts.

Eighteen to twenty-four hours of grinding is sufficient in most cases to produce a satisfactory disruption of staphylococci, typhoid and pertussis organisms.

DETERMINATION OF FIBRIN BY THE BIURET METHOD‡

JOSEPH FINE, M.D., D.P.H., B.Sc., LONDON, ENG.

IT HAS been shown (Fine, 1935) that the albumin and globulin of serum can be estimated rapidly and satisfactorily by an application of the biuret technic. A successful extension of the method to the proteins of plasma clearly must depend on whether fibrin can likewise be estimated. This was considered probable when it was found that fibrin yields a violet solution with $CuSO_4$ and NaOH, which in the colorimeter matches perfectly the color yielded by serum proteins.

Methods of Estimating Fibrin in Plasma.—These methods fall into three main groups: the Kjeldahl (Cullen and Van Slyke, 1920); gravimetric (Gram, 1921; Foster and Whipple, 1921); and colorimetric (Wu, 1922). In all these methods the fibrin is first separated from the plasma by recalcification with $CaCl_2$, though Howe (1923) in another Kjeldahl method precipitates the fibrinogen with 10.6 per cent Na_2SO_4. Of the above methods that of Wu was most suitable for rapid clinical purposes, but it is suggested that the biuret technic will now be the method of choice.

Biuret Determination of Fibrin.—This was carried out on a sample of commercial fibrin and on several samples of human plasma, the results being compared with gravimetric determinations. The standard for the biuret method was a 0.24 per cent serum protein solution, as previously described in the method for serum proteins.

Commercial Fibrin: The biuret method was carried out by dissolving a weighed amount in 1 ml. of hot 30 per cent NaOH and making up the cooled solution to 10 ml. with distilled water and 1 ml. of 5 per cent $CuSO_4$. The mixture was shaken for ten seconds and the supernatant fluid after centrifuging was compared with the violet solution obtained from the standard. The

*Manufactured by Strom Steel Ball Company, Cicero, Illinois.
†Manufactured by Burgess-Parr Company, Moline, Illinois.
‡From the Laboratory of Chemical Pathology, St. Bartholomew's Hospital.
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gravimetric determination was made by heating a weighed amount at 110° C. to constant weight and then ashing. The weight of ash + moisture was subtracted from the weight of the sample and the difference expressed as a percentage of the sample.

Plasma Fibrin: The first stage in both the biuret and the gravimetric determinations was the recalcification of the oxalated plasma by the method of Cullen and Van Slyke (1920), 2 c.c. of plasma being added to 60 c.c. of 0.85 per cent NaCl, and 2 c.c. of 5 per cent $CaCl_2.6H_2O$ (or 2.5 per cent anhydrous $CaCl_2$), and the clot which formed in one-half to one hour collected in the usual way on a glass rod, slipped off and washed in distilled water. From this stage the biuret determination was carried out in the same way as for commercial fibrin. In the case of the gravimetric determination, the dry weight and the weight of the ash were first obtained: the weight of the ash (CaO) was then multiplied by $\frac{16}{7} = \frac{CaC_2O_4}{CaO}$ and the result subtracted from the dry weight to give the weight of actual fibrin. This calculation, not elsewhere suggested, is clearly necessary since the Ca which presumably is combined as oxalate in the dried fibrin is converted into oxide during ashing. The calculation is not required for commercial fibrin owing to the small amount of ash present (½ per cent); the fibrin of recalcified plasma, however, may contain 10 or 20 per cent ash owing to the incorporation in the clot of calcium oxalate derived from interaction between excess $CaCl_2$ and excess potassium oxalate. Failure to make the above calculation gave grossly high results for the fibrin of normal blood, a consequence theoretically to be expected. When, however, the calculation was made, not only did the resulting gravimetric figures fall within normal limits, but they also agreed very closely with the figures given by the biuret method.

TABLE I

RESULTS

SAMPLE	PER CENT FIBRIN	
	GRAVIMETRIC	BIURET
Plasma 1	—	0.288
2	0.40	0.38
3	0.32	0.32
Commercial fibrin	90.0	85.0

Technic for Estimation of Plasma Proteins.—

1. Total Proteins.
2. Albumin.

} The procedure is identical with that elsewhere described for serum proteins (Fine, 1935).

3. Fibrin: 2 c.c. plasma is recalcified by the method of Cullen and Van Slyke (1920) outlined above. The fibrin yielded is dissolved by heating in 1 c.c. 30 per cent NaOH in a 15 c.c. centrifuge tube, and the estimation is continued as for total proteins.

4. Standards: The standard required for total proteins and albumin is prepared from 5 c.c. of a 0.24 per cent serum protein solution. Unless a high fibrin value is expected a second standard should be prepared from 2.5 c.c. of the above solution.

Calculation: If the unknown is set at 20 mm., and the readings of the standard for total protein, albumin, and fibrin are X, Y, Z, respectively, then

Total protein	= 0.3X per cent
Albumin	= 0.24Y per cent
Fibrin	= 0.03Z per cent, using 5 c.c. standard
	or 0.015Z per cent, using 2.5 c.c. standard
Globulin	= [0.3X − 0.24Y − 0.03Z] per cent
	or [0.3X − 0.24Y − 0.015Z] per cent

SUMMARY

The estimation of fibrin by the biuret method, using serum protein as standard, gives results closely agreeing with those by the gravimetric method. A technic is outlined for the estimation of plasma proteins by the biuret method, which is considered the method of choice for clinical purposes.

I am indebted to Dr. G. A. Harrison for permission to carry out the above investigation in his laboratory.

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A SATISFACTORY DRINKING FOUNTAIN FOR CAGED ANIMALS*

GEORGE WALKER, M.D., BALTIMORE, MD.

THE accompanying cut shows an apparatus for supplying fresh, clean water to animals in cages. The principle on which it works is an old one.

It consists of an ordinary six-ounce wide-mouthed bottle (Fig. I, *A*), a perforated cork (Fig. I, *B*), a curved glass tube (Fig. I, *C*), a metal water

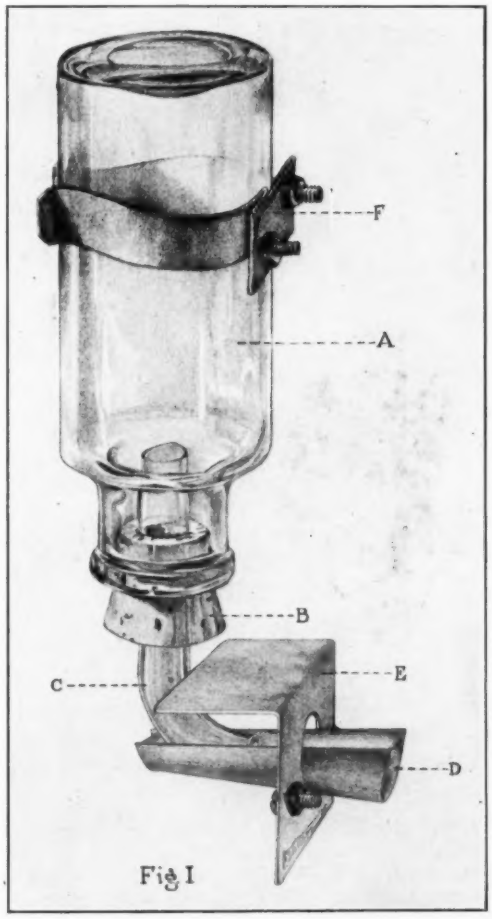

Fig. I

trough (Fig. I, *D*) held in place by a metal holder (Fig. I, *E*), while the bottle, in turn, is held on the outside of the cage by a spring clamp (Fig. I, *F*), which is fastened to the exterior of the cage by two screws. The metal holder which supports the trough and tube is attached to the cage by two screws and the end of the trough extends through a hole cut in the wire with a pair of clippers. This

*Received for publication, August 6, 1935.

hole is made sufficiently large to accommodate the trough, but not large enough for small animals to get through. The end of the trough which extends into the cage is deeper than the end on which the tube rests. This arrangement insures a proper flow of water.

As the animal drinks, the water in the trough sinks below the level of the upper edge of the glass tube and allows air to bubble up and water in turn to run down.

The bottle is easily detached from the cage and may be washed and filled daily.

A larger trough (Fig. II) is made for animals such as rabbits, guinea pigs, and fowls.

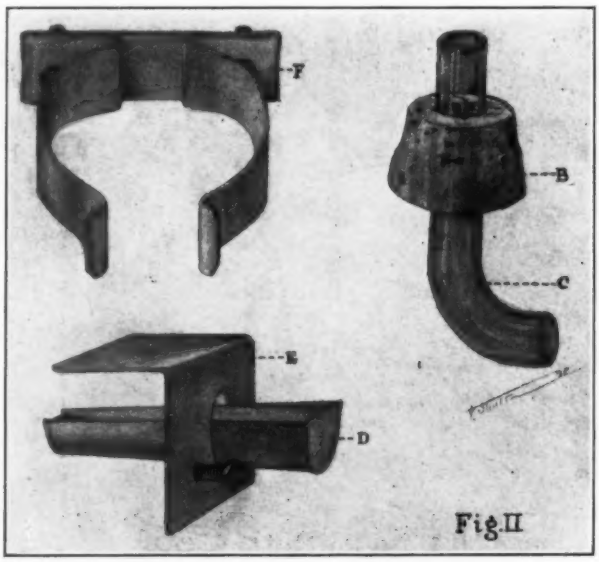

Fig. II

The advantages are:

1. This apparatus insures clean fresh water.
2. The trough is placed about two inches from the floor of the cage and is thus protected from becoming contaminated with dirt and trash.
3. The animal drinks with ease.
4. The bottle is always visible so that one can tell at a glance whether the cage is being supplied with water.
5. Water can be supplied without opening the cage.

This complete drinking outfit can be made with little trouble or can be procured from George H. Wahmann, 18 North Greene Street, Baltimore, Maryland.

1 East Center Street

MODIFICATION OF FOLIN AND WU'S METHOD FOR SUGAR DETERMINATION*

MELVILLE SAHYUN, PH.D., DETROIT, MICH.

THE method for blood sugar determination described in this paper is a modification of Somogyi's[1] and Folin and Wu's.[2] Somogyi was first to suggest the use of 10 per cent sodium tungstate and 7 per cent copper sulphate solutions for the precipitation of blood proteins. When these reagents were tried on tissue proteins[3] the concentration of copper sulphate was found inadequate, for the filtrates so obtained were not completely protein-free. Consequently the concentration of copper sulphate was increased to 10 per cent.

In an earlier paper by the author[4] it was pointed out that the addition of 0.25 M sodium sulphate to the alkaline copper sulphate reagent of Folin and Wu increased the stability of the final blue color. This reagent was adopted.

The various reagents required are as follows:

REAGENTS

Ten Per Cent Copper Sulphate.—Dissolve 100 gm. of copper sulphate crystals in about 900 c.c. of distilled water. Heat to boiling until the copper sulphate is dissolved. Let stand overnight, filter, and dilute to *one liter.*

Ten Per Cent Sodium Tungstate.—Prepare a 10 per cent solution in the usual manner.

Alkaline Copper Sulphate.—Dissolve 50 gm. of anhydrous sodium carbonate in about 500 c.c. of water containing 0.25 M sodium sulphate. Add 7.5 gm. of tartaric acid. Shake until the latter is dissolved. Add 4.5 gm. of pure copper sulphate crystals. Mix. When the latter is dissolved dilute to *one liter.* Allow the mixture to stand for several days and filter. The filtrate must be perfectly clear.

Phosphomolybdic Acid.—Introduce into a two-liter beaker 70 gm. of pure molybdic acid (ammonia free) and 10 gm. of sodium tungstate. Add 800 c.c. of 5 per cent sodium hydroxide. Boil vigorously for thirty minutes. Cool. Add about 100 c.c. of distilled water and 250 c.c. of concentrated phosphoric acid (85 per cent). Dilute to *one liter.*

Note: Special care should be taken in thoroughly cleaning the glassware for the preparation of these reagents.

Standard Sugar Solution.—Prepare a stock solution of 1 per cent anhydrous glucose of the purest quality available in a saturated solution of benzoic acid. One cubic centimeter will contain 10 mg. This stock solution keeps indefinitely if well stoppered.

One-Tenth Milligram of Glucose Standard.—Transfer accurately 5 c.c. of the 1 per cent stock glucose solution into a 500 c.c. volumetric flask. Dilute to the mark with a saturated solution of benzoic acid. One cubic centimeter contains 0.1 mg. glucose.

If necessary 0.05 and 0.2 mg. glucose standards may be prepared in a similar manner.

Benzoic Acid.—Transfer 2.9 gm. of benzoic acid into a liter beaker containing 800 c.c. of distilled water. Heat to boiling until the benzoic acid is dissolved. Dilute to *one liter.* Use this benzoic acid solution for the preparation of the various glucose standards.

Glucose standards prepared in the manner described will keep indefinitely.

*From the Biochemical Research Laboratory, Frederick Stearns & Co.
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Precipitation of Blood Proteins.—Transfer 1 c.c. of blood into a flask containing 7 c.c. of distilled water. Add 1 c.c. of 10 per cent sodium tungstate and mix. Add 1 c.c. of the 10 per cent copper sulphate and shake vigorously. The 10 per cent copper reagent must always be added last. Allow the mixture to stand for ten minutes before filtration.

METHOD

Pipette 2 c.c. of the protein-free filtrate into a 25 c.c. Folin sugar tube and to another tube pipette 2 c.c. of the glucose standard (0.1 mg. per 1 c.c.). In the event that blood sugar is low, use the substandard of 0.05 mg. simultaneously. To each tube add 2 c.c. of the special alkaline copper sulphate. Transfer

TABLE I

BLOOD SUGAR DETERMINATIONS

SAMPLE	FOLIN AND SVEDBERG METHOD[5]	DESCRIBED METHOD
	MG. PER 100 C.C. OF BLOOD	MG. PER 100 C.C. OF BLOOD
	MG.	MG.
1	99	95
2	110	110
3	89	89
4	74	77
5	111	111
6	106	109
7	97	95
8	85	83
9	148	148

TABLE II

THE ESTIMATION OF BLOOD SUGAR BY BENEDICT'S METHOD AND BY THE METHOD DESCRIBED

SAMPLE	MG. BLOOD SUGAR PER 100 C.C.	
	BENEDICT'S[6]	DESCRIBED METHOD
	MG.	MG.
1	125	122
2	104	108
3	103	102
4	115	114
5	89	88
6	83	78
7	91	87
8	108	105

TABLE III

DETERMINATION OF BLOOD SUGAR BY FERMENTATION

The blood glucose was subjected to yeast fermentation and the "saccharoids" in the filtrates were determined. The difference between the reducing substances in the unfermented sample of blood and in the same sample after fermentation is considered fermentable sugar.

Reducing and nonreducing substances in blood, mg. per 100 c.c.

SAMPLE	TOTAL REDUCING SUBSTANCES	FERMENTABLE SUGAR	NONFERMENTABLE SUGAR
	MG.	MG.	MG.
1	83	77	3
2	121	116	5
3	83	81	2

the tubes to a boiling water-bath and boil for six minutes. Remove and cool for three or four minutes in a water-bath. Add to each 2 c.c. of the phosphomolybdic acid reagent and five minutes later dilute to the 25 c.c. mark. Mix by inverting the tubes 3 times. Compare colors.

CONCLUSION

The main feature of the method described is the stability of the blue color developed upon the addition of the phosphomolybdic acid reagent. This improvement permits the simultaneous determination of a large number of samples. Furthermore, it is believed that the estimation of blood sugar by this method is close to the true sugar content of blood. This assumption is based not only on a comparative determination of the blood sugar by such standard methods as Benedict's and Folin and Svedberg's methods, but also by the fermentation method described by Somogyi.[7]

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A RAPID METHOD FOR ROUTINE SERUM PROTEIN DETERMINATION*

RUSSEL O. BOWMAN, PH.D., PROVIDENCE, R. I.

MOST hospital laboratories, where serum protein determinations are done, use the Howe[1] method or some modification of it. Because this method is time-consuming and somewhat technical, there are many hospitals where serum protein determinations are not part of the laboratory routine. There is great need for a rapid, accurate method for serum protein estimation, so that this information may be available to more clinicians as an aid in diagnosis and treatment of renal, cardiac, hepatic, and nutritional disturbances.

In 1920, Denis and Ayer[2] published a method for determination of cerebrospinal fluid protein. This method can be applied to the determination of serum or plasma proteins, and can also be used, after sodium sulphate separation, to determine the albumin and globulin fractions present. The method has been used at this hospital for over a year with success.

REAGENTS

1. Protein Stock Standard Solution: 20 c.c. of fresh clear human serum are diluted to 200 c.c. in a volumetric flask with 15 per cent sodium chloride solution. Mix and filter. Add a few crystals of thymol and the solution will keep for six months or more in the ice chest.[3] Total nitrogen of this filtrate is determined by the micro-Kjeldahl method and nonprotein nitrogen is determined on a sample of the original serum by the Folin-Wu method.[4] The total nitrogen of the filtrate minus one-tenth of the nonprotein nitrogen of the serum gives protein nitrogen, and protein nitrogen multiplied by 6.25 gives the protein content of the filtrate.

2. Protein Standard Solution: The stock standard is diluted with distilled water so as to contain 30 mg. per 100 c.c. of solution. This standard keeps for one year when preserved with thymol and kept in the ice chest.

3. 5 per cent Sulphosalicylic Acid: Dissolve 50 gm. of C. P. sulphosalicylic acid in distilled water and make up to a liter. Filter. This keeps indefinitely.

4. 22.2 per cent Sodium Sulphate: 111 gm. of anhydrous C. P. Na_2SO_4 are dissolved in distilled water with the aid of heating to 37° C. in a 500 c.c. volumetric flask. The volume is made up to the mark at this temperature. With purified salt, filtering is not necessary. This reagent is kept at 37° C. in the incubator to prevent crystallization of the salt at room temperature.

5. 0.9 per cent Sodium Chloride.

METHOD

Exactly 0.2 c.c. of blood plasma or serum is diluted by adding 39.8 c.c. of normal saline from a buret. If the determination is to be run at once, distilled water may be used instead of the saline.

*From the Laboratories of Rhode Island Hospital.
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After thorough mixing, 2 c.c. of the diluted serum is pipetted into a test tube and 2 c.c. of standard protein solution is pipetted into a similar tube. To each add 2 c.c. of 5 per cent sulphosalicylic acid and mix thoroughly by rotating the tubes. Compare in the colorimeter.

Calculation: R × 6 = gm. protein per 100 c.c. where R is the ratio of depth of standard to depth of unknown solution.

For albumin determination the globulins are precipitated according to the Howe[1] method, one volume of serum to 30 volumes of 22.2 per cent sodium sulphate let stand for four hours or more in the incubator and filtered until clear. To 1 c.c. of the filtrate add 3 c.c. of distilled water and 4 c.c. of 5 per cent sulphosalicylic acid. Mix and compare with a standard as above.

Calculation: R × 3.72 = gm. albumin per 100 c.c.

I have used a Hellige microcolorimeter which has a revolving dial graduated to read directly the ratio of depth of standard solution to depth of unknown solution looked through. This colorimeter is especially adapted to use of small quantities. For other colorimeters the amounts of unknown and standard should be increased, and for those where the depth of solution looked through is more than 1.5 cm., when the plunger is midway immersed in the cup, it will be necessary to dilute total protein, albumin and standard tubes 1:1 with distilled water before adding sulphosalicylic acid. This gives less turbidity and allows more accurate matching. If unknowns are less than one-half or more than twice as turbid as the standard tube they should be made up again, diluting so that they approximate the standard, and appropriate factors used in the calculation. Too concentrated protein solutions flocculate on adding sulphosalicylic acid.

TABLE I

COMPARISON OF SULPHOSALICYLIC ACID AND MICRO-KJELDAHL METHODS FOR PROTEIN DETERMINATION

CASE	MATERIAL	AGE	DIAGNOSIS	SULPHOSALICYLIC ACID GM. PER 100 C.C.		N.P.N.	MICRO-KJELDAHL GM. PER 100 C.C.	
				TOTAL	ALBUMIN		TOTAL	ALBUMIN
F. A.	Serum	26	Bile peritonitis	3.90	2.34	49	4.00	2.52
Pooled	Serum	--	Malnutrition	4.20	----	42	4.60	----
A. R.	Serum	44	Chronic nephritis	5.40	2.72	78	5.54	2.85
M. O.	Plasma	44	Pyelonephritis	5.85	2.57	94	5.98	2.88
Pooled	Serum	--	----------	5.98	----	32	5.96	----
Pooled	Serum	--	----------	6.15	----	27	6.38	----
Pooled	Serum	--	Jaundice	6.69	4.22	29	6.62	3.90
L. M.	Plasma	61	Ménière's syndrome	7.02	4.97	25	6.93	4.56
Pooled	Serum	--	Normal	7.26	4.41	36	7.26	4.37
C. R.	Serum	40	Paget's	8.04	----	30	7.78	----

TABLE II

COMPARISON OF SULPHOSALICYLIC ACID AND GRAVIMETRIC METHODS FOR PROTEIN DETERMINATION

MATERIAL	SULPHOSALICYLIC ACID GM. PER 100 C.C.	GRAVIMETRIC GM. PER 100 C.C.
Serum	7.65	7.69
Serum	7.23	7.25
Plasma	6.44	6.62
Plasma	6.29	6.16

RESULTS

Table I gives a few comparative analyses of blood sera and plasmas using the present method and the Howe[1] method (micro-Kjeldahl digestion with Superoxyl for final stage, and direct nesslerization). Nonprotein nitrogens were run by the Folin-Wu method.[4] All figures are the average of duplicate and

occasionally triplicate determinations. Check determinations showed better agreement by the sulphosalicylic acid method than by the digestion method, proving that there is less chance for technical error in the present method.

Table II gives a further comparison of the sulphosalicylic acid and gravimetric methods.[5] These two methods agree quite well and the gravimetric method is considered the most accurate determination of serum protein yet devised.

Because of the relation of serum protein to serum calcium concentration and the necessity of determining calcium, inorganic phosphorus, and total protein on the smallest possible amount of serum, the method has been applied for determination of all three on a sample of 2 c.c. of serum. Calcium and inorganic phosphorus were determined according to Lowenberg and Mattice.[6] The small amount of supernatant fluid from calcium precipitation which remains after part is used for phosphorus determination, is sufficient for protein estimation. Two-tenths cubic centimeters of this supernatant fluid, which is a 2:5 dilution of the serum, is diluted with 15.8 c.c. of distilled water or saline from a buret so as to make a 1:200 dilution of the serum. This is compared with a standard as given above for total protein estimation.

A series of tests were run using 0.2 per cent ammonium oxalate, 0.9 per cent saline and weak buffer solutions of phosphates of pH 6.8 to 9.0 as diluting fluids before sulphosalicylic acid was added. Results were alike for the different diluents used. When distilled water is used to dilute certain sera or plasmas, especially when diluted and let stand, there is a partial precipitation of globulin. This can be redissolved by adding a few crystals of sodium chloride to the tube and mixing, and without any effect on the final result. When determination is done right after dilution, saline or distilled water may be used as a diluent without affecting the value obtained.

Publication of this article has been delayed for a year because on two different samples it was not possible to check the micro-Kjeldahl and sulphosalicylic acid procedures. In each of these cases the sulphosalicylic acid method gave much lower figures than the digestion method. Lipemia or Bence-Jones proteinemia was not present. Further work to explain the discrepancy was impossible since in each case the sample was obtained shortly before death of the patient. Several more checks have been obtained by the two methods and no other puzzling results have been obtained.

DISCUSSION

It has been shown that oxalate present in oxalated plasma does not affect the determination of protein by this method. Moderate changes in pH of the diluted sample do not change the results. Because of the large dilution factor, any changes present in pathologic bloods will have little or no effect on the final result. The method seems to be applicable to all sera or plasmas and suitable for routine work in a hospital laboratory.

It is interesting to note that the cost to the hospital of the sulphosalicylic acid method is about one-tenth the cost of the digestion method. Total protein determinations can be run at a cost of about five cents each, including labor, materials and chemicals.

The most important source of error in the present method is in measurement of the initial sample for dilution. Any error here is multiplied many times by the dilution factor. The satisfactory results above were obtained by delivering between marks on a 1 c.c. Exax pipette graduated in 1/100 c.c. Other errors to be avoided are sedimentation of the precipitated protein in too concentrated solutions, incomplete mixing of diluted unknowns, and unequal light to the colorimeter cups.

SUMMARY

The Denis-Ayer[2] method for quantitative determination of protein in cerebrospinal fluid has been applied to blood serum or plasma protein estimation.

This method, with care, is as accurate as micro-Kjeldahl determination with nesslerization, or a gravimetric procedure. It can be used for the determination of albumin and globulin fractions of blood serum or plasma protein.

A method is given for determination of calcium, inorganic phosphorus and total protein on 2 c.c. of blood serum.

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A METHOD FOR THE MICRODETERMINATION OF PROCAINE IN CEREBROSPINAL FLUID*

HARRY KOSTER, M.D., F.A.C.S., ARTHUR SHAPIRO, M.D., AND EDNA POSEN, M.A.,
BROOKLYN, N. Y.

IN WORK concerning the fate of procaine in spinal fluid, several methods for determining procaine were tried but none was found satisfactory. Two different types of methods are available for this determination. The first is based on the color given by the diazo reagent.[1-3] This procedure involves a theoretical error since p-amino-benzoic acid, whose physiologic properties are different from procaine and which might be split off from the procaine molecule, develops the same color with these reagents. The second type is based on general alkaloid reactions.[4-7] (For complete bibliography see.[3]) Using large amounts of procaine these methods may give accurate results but the amounts of procaine in small samples of spinal fluid (0.2 to 1.0 mg.) cannot be determined with such reagents.

In this paper we describe a new method based on the fact that procaine and vanillin in dilute H_2SO_4 give rise to a colored compound (Maesmaeker and Lucas[8]). This compound, we found, may be precipitated quantitatively with potassium mercuric iodide and after redissolving, determined colorimetrically. Since p-amino-benzoic acid under the conditions described below is not precipitated by these reagents, the error due to the presence of this substance is eliminated.

EXPERIMENTAL

Reagents.—1. Vanillin reagent: Dissolve 10 gm. of vanillin in 40 c.c. of 95 per cent alcohol. Make up to 1 liter with N/12 sulphuric acid and filter if necessary.

2. Potassium mercuric iodide reagent: Dissolve 40 gm. of HgI_2 and 60 gm. of KI in a liter of water.

3. 0.1M sodium phosphate solution: Dissolve 38.02 gm. of $Na_2PO_4 + 12H_2O$ in water and make the volume up to 1,000 c.c. Filter if necessary. This solution should be made up fresh every few days.

4. Approximately 0.05M phosphoric acid: Calculate approximate number of cubic centimeters of syrupy phosphoric acid (85 or 90 per cent) necessary to make up a liter of 0.05M H_3PO_4.

5. 0.080 per cent K_2CrO_4 solution.

Apparatus.—No special apparatus is required except: (1) Colorimeter with micro cups and plungers, 20 mm. depth with 1 c.c. fluid. (2) Eastman Kodak color filters Nos. 35 and 47 which are used together in the eyepiece of the colorimeter.

Method.—An amount of fluid containing 0.2 to 1.5 mg. of procaine is delivered into a 15 c.c. conical centrifuge tube. A little practice in judging the amount of precipitate formed will make it easy to choose samples. Ordinarily during the course of anesthesia this lies between 0.05 and 0.5 c.c. To this is added about 1 c.c. of vanillin reagent and shortly after, about 1 c.c. of potassium mercuric iodide reagent. These reagents are conveniently added from dropping bottles. After standing a few minutes the precipitates are centrifuged for about five minutes at moderate speed. The supernatant fluid is then decanted as completely

*From the Richard Morton Koster Research Laboratory of the Crown Heights Hospital.
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as possible and the yellow oily precipitate on the sides and bottonf of the tube is rapidly washed with three successive portions of distilled water. It is important to wash out most of the H_2SO_4 clinging to the precipitate. The tubes are dried at room temperature, preferably in a dessicator.

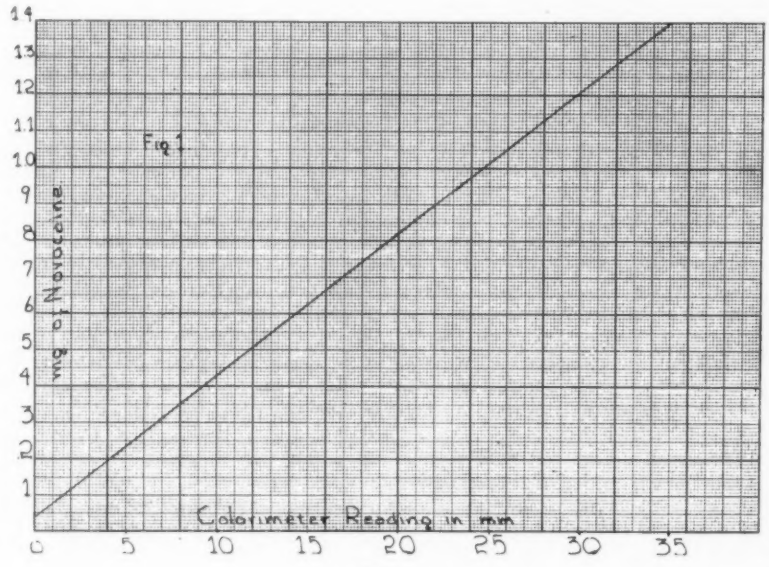

Fig. 1.

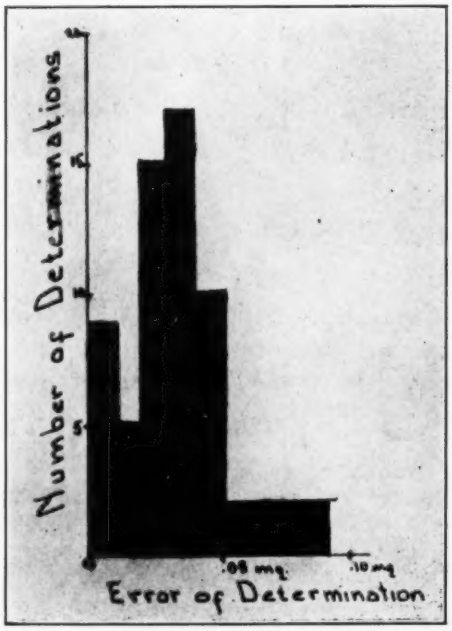

Fig. 2.

To the dried precipitate in the centrifuge tube 3 c.c. of 0.1M Na_3PO_4 are added and the solution stirred vigorously with a glass rod for one minute. One cubic centimeter of 0.5M H_3PO_4 is then added and the colored solution is then transferred rapidly to one of the cups of the microcolorimeter and read against 0.08 per cent K_2CrO_4, using both color filters together in

the eyepiece. The color should be read about two minutes after the addition of the Na_2PO_4 but it is safe to read within five minutes. In a series of determinations, the cup and plunger are rinsed with the new solution each time.

Calculation of Results.—The amounts of novocaine may be read from the graph in Fig. 1 or may be calculated from the formula:

$$0.040 + 0.039\ X = \text{Mg. procaine in Sample of Unknown}$$

(X = Color reading in mm. of standard against 10 mm. of unknown)

DISCUSSION

The average error in 64 determinations of unknown solutions ranging from 0.2 mg. to 1.0 mg. was 0.027 mg. The most satisfactory results were obtained with samples of 0.4 to 0.6 mg. (see Fig. 2).

Other constituents of spinal fluid do not interfere with this reaction. If the reagents are added to procaine-free spinal fluid, only a faint turbidity develops, which after centrifuging and redissolving gives no color that would interfere with the determination. Known amounts of procaine added to spinal fluid are recovered within the same limits of error as from aqueous solution. The volume of spinal fluid does not seem to affect the completeness of the reaction. The same results were obtained in a sample of spinal fluid when precipitated directly or after dilution from 0.2 to 4 c.c.

P-amino-benzoic acid, which may be a split product of procaine, gives a yellow color with the reagents and may precipitate as yellow crystals if the sides of the tube are scratched with a glass rod. If scratching is avoided, no precipitate is formed and procaine in amounts from 0.2 to 1.0 mg. may be correctly determined even in the presence of as much as 2.5 mg. of p-amino-benzoic acid.

Several other local anesthetics such as anesthesin and butyn give similar reactions. Though we made no special investigation of these substances, it was noted that the color given by anesthesin is much weaker than that given by procaine.

The reaction can also be used to distinguish qualitatively between cocaine and procaine since cocaine gives a colorless precipitate.

SUMMARY

A method for the quantitative determination of procaine in spinal fluid based on its reaction with vanillin and potassium mercuric iodide in acid solution has been described. This method has been shown to give reliable results even in the presence of p-amino-benzoic acid.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

RENAL FUNCTION: Urea Ratio as a Measure of, Mosenthal, H. O., and Bruger, M. Arch. Int. Med. 55: 411, 1935.

The urea ratio expressed as the percentage of urea nitrogen in the total nonprotein nitrogen in the blood or $\frac{100 \times \text{urea nitrogen}}{\text{nonprotein nitrogen}}$ is a satisfactory index of renal efficiency. When the Folin-Wu filtrate (laked blood) is used for the determination both of the urea nitrogen and of the total nonprotein nitrogen in the blood, it is noted that the urea ratio varies with renal function as follows:

(A) with normal renal function, an index of 44 or less; (B) with maximal impairment of renal function, an index of 80 or higher; (C) with improvement in renal function, a drop in the ratio, and with progressive impairment of function, a rise in the ratio.

The determination of the urea ratio at intervals measures progressive changes in renal efficiency and affords a reliable index of renal function, regardless of the fluctuations of the total nonprotein nitrogen of the blood. Although the urea nitrogen or the total nonprotein nitrogen in the blood may be at a normal level, the urea ratio reveals renal insufficiency when it exists. This test may be carried out on one specimen of blood; it does not require prolonged observation of the patient or collection of urine. It furnishes a numerical index of the degree of impairment of renal function and is equally applicable to patients in private practice and to those in the hospitals.

SYNOVIAL FLUID, Characteristics of, in Arthritis, Keefer, C. S., Myers, W. K., and Holmes, W. F. Arch. Int. Med. 54: 872, 1935.

One hundred and twenty samples of synovial fluid from ninety patients with various types of arthritis were studied to determine the diagnostic significance of the various biologic and chemical characteristics of the fluid.

All the patients were studied while residing in the hospital. With a needle and syringe, fluid was aspirated from the joints, the usual aseptic precautions being taken. The skin was anesthetized with a 2 per cent solution of procain hydrochloride.

A sample of the aspirated fluid was mixed with sodium oxalate crystals to prevent clotting and examined chemically for the content of total protein, sugar and nonprotein nitrogen. In forty-five cases, blood was withdrawn from a vein simultaneously for comparison of the sugar and nonprotein nitrogen content of the blood and the synovial fluid.

Aspiration was not performed following a period of fasting, so that this factor will require consideration in the interpretation of the comparative results, to be discussed presently.

Total cell counts were done on the synovial fluid immediately after aspiration. Differential cell counts were done according to the supravital technic. Gonococcic complement fixation tests were done on the blood and synovial fluid in a number of cases, and the Wassermann test was likewise done on both the blood and the synovial fluid.

The synovial fluids were cultured for microorganisms, and in suspected cases of tuberculous arthritis samples of the fluid were injected into guinea pigs.

The bacteriologic examination of the fluids yielded information of the greatest value in the etiologic diagnosis of arthritis. Inoculation of guinea pigs with the synovial fluid was helpful in the diagnosis of tuberculous arthritis.

The results of the gonococcic complement fixation and Wassermann tests of the blood and synovial fluids were in agreement. Both tests were of distinct aid in the etiologic diagnosis of disease of the joints.

The total cell count of the synovial fluid was increased in all the types of arthritis studied. It was highest in the infected fluids and lowest in the cases of charcot joints and traumatic arthritis. Most noninfected fluids contained less than 40,000 cells per c.mm.

When the synovial fluid was infected with microorganisms, the polymorphonuclear cells were greatly increased, from 86 to 100 per cent, and the lymphocytes, monocytes and clasmatocytes were few in number. In tuberculosis of the joints, the polymorphonuclear count varied from 46 to 93 per cent, and the lymphocytes and monocytes were increased.

The percentages of lymphocytes, clasmatocytes and monocytes were always higher in the noninfected fluids than in the infected fluids. The presence of a low cell count with an increase in monocytes, lymphocytes and clasmatocytes was an indication of a noninfected fluid.

The chemical examination of the fluids yielded no information of diagnostic value in discriminating between infected and noninfected fluids. The nonprotein nitrogen of both the infected and the noninfected synovial fluids was the same as that of the blood. The sugar content varied with the presence of organisms, the number of cells and the level of the sugar in the blood. A low sugar content did not always mean an infected fluid. The total protein value of the synovial fluid was increased in both groups, and indicated only an inflammatory reaction.

Aside from the bacteriologic, cytologic and serologic examinations of the synovial fluid, other tests yielded little information of diagnostic value.

JAUNDICE, Mechanism of, Elton, N. W. Am. J. Clin. Path. 5: 40, 1935.

Application of the ring test modification of the van den Bergh reaction to the study of jaundice reveals many data inconsistent with prevailing classifications and concepts of its nature. The commonly accepted concept of hemolytic jaundice has been found to be especially fallacious. With these newer data a working hypothesis has been constructed to explain the mechanism of jaundice on the basis of the bilirubin excretion mechanism, disturbances of which are operative in producing it. Data from the work of many investigators have been correlated in this analysis.

The liver is the excretory organ for bilirubin. Bilirubin in the mammal is produced chiefly extrahepatically. Being insoluble in aqueous solvents except alkalis, bilirubin enters the blood stream from its sites of origin in a state of colloidal suspension. The normal alkalinity of the blood is insufficient to change its nascent state. The liver, in excreting bilirubin, converts it into a water-soluble salt, a bilirubinate. Since bilirubin is an acidic pigment, the alkaline bile salt of the liver parenchyma is believed to be the agent which neutralizes the pigment sufficiently to form the salt.

The structural design of the liver lobule, the known affinity of the Kupffer cell for colloidal particles, and a slight modification of the bile secretion mechanism as conceived by Geraudel, lead to the division of the liver lobule, from the standpoint of bilirubin excretion, into two major functional zones and an intermediate zone, as follows:

1. *Acceptance zone,* involving the peripheral portion of the lobule, where bilirubin is taken up from the slow-flowing sinusoidal stream by Kupffer cells and passed on by them into the underlying polygonal cells for conversion and excretion.

2. *Conversion zone,* an intermediate zone midway between the periphery and the center of the lobule, where bilirubin is converted to a water-soluble salt, a bilirubinate, as it passes down the liver cords through the polygonal cells toward the central portion of the lobule.

3. *Excretion zone,* involving the central portion of the liver lobule, where bilirubinate is finally excreted as a bile constituent by the central zone polygonal cells into the bile canaliculi, through which it passes to the periphery and into the ducts.

The nature of jaundice is advisedly considered from the point of view of etiologic factors rather than of classification. Etiologic factors have been postulated as follows: (1)

mechanical obstruction of the bile ducts; (2) dynamic elevation of the excretion threshold; (3) adynamic elevation of the excretion threshold; (4) pigment overload; (5) damage to liver parenchyma; (6) patent ductus venosus.

TUBERCLE BACILLUS, The Progeny of, Maher, S. J. Am. Rev. Tuberc. 31: 350, 1935.

In this paper Maher reports and summarizes the results of his studies on the mutation of tubercle bacilli in laboratory cultures whereby they become cocci and diplococci nonacid-fast and in this form produce an acid harmless to themselves but harmful to the bacilli from which they are derived.

The paper summarizes these studies since their inception in 1908 and describes the method employed to bring about the transformation so that it may be employed by those interested. It has succeeded with all forms of tubercle bacilli: human, bovine and avian and with cultures habituated to growth at room temperature as well as fresh cultures growing only at 37° C.

While mutation can be induced in tube cultures as well as in flask cultures, it is more certain and occurs more rapidly in the latter which are therefore more suitable for the purpose.

Method.—Flask cultures: To a culture of tubercle bacilli on glycerin broth (pH 6.4-7.2) add a volume of sterile litmus milk (prepared from skimmed milk) equal to 25 or 30 per cent of the total volume of glycerin broth. In other words, to a culture having a volume of 250 c.c. from 50 to 60 c.c. of litmus milk is added. The flask is then stored in the dark at room temperature until the litmus blue color begins to change to pink or white, when it is ready for examination. In the case of young cultures incubation for a few hours at 37° C. after addition of the milk will facilitate mutation.

The growth on solid media is scraped off and emulsified in 2 c.c. of sterile plain broth and the emulsion poured into 250 c.c. of glycerin broth. Then add 50 c.c. of sterile litmus milk and incubate as described.

The course of events is somewhat as follows: The first change, apparent usually within 4 or 6 days, is a whitening of the color of the medium in its upper layers. Examination of smears at this time shows, in addition to granular acid-fast rods, free red and blue granules and many blue (i.e. nonacid-fast) cocci and diplococci. When mutation has been complete and no acid-fast forms are found, the cocci and diplococci may be transplanted to other media upon which they grow readily.

Sometimes mutation is slow and even when the acid production is evidenced by the formation of curds and whey in the flask only tiny, nonacid-fast rods will be seen in the smears. If these are subcultured and incubated at 37° C., however, transformation into cocci and diplococci is prompt.

The various changes in the appearance of the transmuting organisms is described in the paper in detail.

Whether or not the fact that the transmuted forms which, by virtue of their acid production, produce a condition inhibitory to the growth of tubercle bacilli, may have any practical application to the treatment of tuberculosis remains for future studies to develop.

TUBERCULIN REACTIONS: Evaluation of White Blood Cell Picture in Ambulatory Child With Positive, Smith, C. H. Am. J. Dis. Child. 49: 318, 1935.

In a group of twenty-three ambulatory patients with the childhood type of tuberculosis, the white blood cell count was examined periodically with the supravital technic in an endeavor to discover any significant alterations produced by manifest disease.

Since differential percentages vary with age, the results were separated into two groups and compared with similar blood counts of normal children. The age periods extended from infancy to four years and from four to thirteen years.

The peripheral blood of the ambulatory child who was already infected but whose contact with the source of exposure had been broken differed, except for minor alterations, in no wise from the normal. In general, a lymphocytic increase, indicative of resistance, constituted the prominent feature.

The blood count does not perform the function of a diagnostic test in tuberculosis. In periods of suspected clinical activity, however, it at times supplies information of a more precise nature as to the status of the pathologic process than can be obtained from the complaints of the patient, the physical examination, or the roentgenogram.

While the cellular changes apply to all age groups, they were most pronounced during activity in the younger child. In the few observations made in this study, the alterations noted in the blood smear differed in degree only from a group of fatal cases of tuberculosis in young children that were studied concurrently.

A tentative monocytic percentage and monocyte-lymphocyte ratio have been suggested to serve as a guide to early manifestations of activity.

Modifications in the white cell picture of the peripheral blood induced by a positive tuberculin reaction require more extended study. It is still problematic whether a comparison of the changes in the blood before and after administration of tuberculin can be utilized to represent the state of focal activity. These changes, however, may possibly serve as an indication of the immunologic status established by the primary infection, and to portray the character of the cellular response in the event of later reinfection.

TUBERCULOSIS, Renal, Incidence of, Greenberger, M. E., Wershub, L. P., and Auerbach, O. J. A. M. A. **104:** 726, 1935.

An attempt has been made to correlate the incidence of renal tuberculosis in 500 cases of pulmonary and extrapulmonary tuberculosis.

Statistics reveal the high incidence of bilateral renal tuberculosis.

The nondestructive, closed or miliary lesion is common.

The destructive, open, organ or chronic surgical tuberculosis of the kidney is notably deficient in symptoms despite the advanced renal involvement.

Pulmonary tuberculosis appeared as the primary focus of infection.

JAUNDICE, A Review of Experimental Investigations, Mann, F. C., and Bollman, J. L. J. A. M. A. **104:** 371, 1935.

An analysis of 214 cases was made in which the concentration of the bilirubin was 2.0 mg. per hundred cubic centimeters of serum or greater, and in which the van den Bergh reaction was indirect. In 138 cases, hemolytic disease was absent; in seventy-six cases it was present.

Increased concentration of bilirubin in the cases in which hemolytic disease is absent means, in the authors' opinion, hepatic dysfunction. The dysfunction has been found to be either constitutional or secondary to hepatic injury. In hemolytic disease, excessive hemolysis is the accepted explanation of the increased concentration of bilirubin. The almost universal presence of some degree of hepatic injury associated with hemolytic disease suggests, however, that hepatic injury and dysfunction may also be partly responsible for elevation of the concentration of bilirubin.

The indirect reaction supposedly rules out the presence of hepatic injury. Actually, however, bona fide hepatic disease has been demonstrated in many cases of this series. Moreover, the authors have witnessed the van den Bergh reaction change from direct to indirect during convalescence from the hepatic injury while increased concentration of bilirubin was still present. The concentration of bilirubin subsequently returned to normal. They have also seen increased concentrations of bilirubin in serum which never had given a direct van den Bergh reaction return to normal concentration as the process of reparation in the liver proceeded. For these reasons, it appears that an indirect reaction in serum containing bilirubin in amounts more than normal does not always mean hemolytic disease and in fact may definitely point to associated hepatic injury.

Some patients who complain of being bilious actually have slight hepatic dysfunction. The dysfunction may be constitutional or secondary to hepatic injury. It is not clear that

all symptoms actually are due to the hepatic dysfunction, but it is probable in some instances that the hepatic dysfunction may be only one of the manifestations of a widespread sympathetic or toxic reaction.

The evidence strongly suggests that among patients who have constitutional dysfunction of the liver, at least of a degree great enough to produce jaundice, disease of the gallbladder is prone to develop. Of the cases studied, in 29 per cent cholecystic disease already has developed. Many of the remaining patients are young. The percentage may increase as these patients grow older.

The mechanism for secretion of bilirubin is a sensitive one. It is influenced by emotion and by disturbances of the sympathetic nervous system, as well as by very minor degrees of hepatic injury. An increased concentration of bilirubin may be the one laboratory evidence of dysfunction and it should be used more widely. It will in all probability disclose many unsuspected cases of mild functional and organic disturbances of the liver as well as a group of individuals of a constitutional type especially susceptible to disease of the gallbladder.

RENAL FUNCTION, Significance of 'Phthalein Test for, Mackay, E. M., and Rytand, D. A. Arch. Int. Med. **55**: 131, 1935.

The significance of the phenolsulphonephthalein test of renal function and, secondarily, of the blood urea concentration was determined by a statistical analysis of the variables in relation to the Addis ratio as a measure of the amount of functioning renal tissue in a group of patients with Bright's disease.

Judged by their correlation and by the shape of their regression line with the ratio, the phenolsulphonephthalein test is superior to the blood urea concentration as a measure of the amount of functioning renal tissue.

For certain clinical purposes the Addis ratio may be predicted as a per cent of normal from the blood urea by dividing 1,755 by the blood urea concentration and from the phenolsulphonephthalein test by multiplying the percentage of the dye excreted by 1.

The odds are 2 to 1 that the ratio may be predicted with a range of ± 17 per cent by means of the phenolsulphonephthalein test and of ± 19 per cent by means of the blood urea concentration.

Considering the individual observations, it is shown that both the blood urea concentration and the excretion of phenolsulphonephthalein may be well within normal limits until at least half the normally functioning renal tissue has been destroyed.

INTESTINAL FLORA, in Newborn Infants, Hall, I. C., and O'Toole, E. Am. J. Dis. Child. **49**: 390, 1935.

An intensive study of sixty-three specimens of meconium and feces secured from ten newborn infants during the first ten days of life showed that while seven passed sterile specimens on the first day, bacteria were present in all specimens by the second day. At least five genera and eighteen species were represented, comprising *M. albus, M. candidus, M. epidermidis, Micrococcus ureae, Micrococcus varians, Micrococcus roseus, Str. faecalis, Str. mitis, Str. cardio-arthritidis, Bact. coli, Bact. paracoli, Bact. aerogenes, Bact. paratyphosum B, Kopfchenbakterien, B. Difficilis* (new species), hay bacillus, *L. acidophilus,* and *L. bifidus.*

Among these, *M. albus, M. candidus, M. epidermidis, Str. faecalis, Str. mitis, Bact. coli, Bact. aerogenes, Kopfchenbacterien,* and the lactobacilli appeared at some time during the first ten days in more than 50 per cent of the infants studied and may be regarded as the most common early bacterial invaders of the intestinal tract of man.

The repeated finding of a new species of pathogenic anaerobe, *B. difficilis,* in the specimens of four of ten infants during the first ten days of life suggests that it may be interesting to study the reported appearance of occult blood in the feces of the newborn, the initial diarrhea sometimes observed in newborn infants and selected cases of convulsions of unexplained origin in infants, with special reference to the occurrence of this organism in the feces.

TISSUE: Butyl Alcohol and Cytological Technic, Zirke, C. Science 80: 481, 1934.

The following mixture is very useful for cleaning slides.

Water	1 part
Xylene	1 part
N-butyl alcohol	1 part
Ethyl alcohol	2 parts

A few drops of n-butyl alcohol added to clouded xylene or alcohol in tissue staining jars will keep the solutions clear and usable.

If the following constitute the original series no precipitate occurs and the solutions remain good for nearly 100 slides.

1. Xylol, 100 per cent.
2. Xylol, 95 per cent, n-butyl alcohol 5 per cent.
3. Absolute ethyl alcohol 90 per cent, n-butyl alcohol 10 per cent.
4. Absolute ethyl alcohol 100 per cent, etc.

PROTEUS X-19, Pleo-Antigenicity of, Welch, H., Mickle, F. L., and Borman, E. K. Am. J. Pub. Health 24: 1157, 1934.

The morphologic and biochemical characteristics of 19 diagnostic Proteus X strains and 5 variants are reported in detail. Marked differences in these characteristics were not noted except for the finding of 13 motile strains in the diagnostic group.

Heterologous antigenic components in common with the Shigella, Eberthella, and Salmonella groups were shown in 11 Proteus X strains. Although these were not demonstrable by direct agglutination in heterologous sera, they were demonstrated in the antisera produced by them on injection into rabbits or failing this, by their ability to absorb heterologous agglutinins from other Proteus sera containing them. Only one of the strains for which sera were prepared showed the entire absence of heterologous components.

The heterologous agglutinins present in Proteus X sera were shown to be bacterial group agglutinins.

Known variants of Proteus X strains were shown to be practically inagglutinable in sera from clinical cases of Rocky Mountain spotted fever and, in addition, certain of these variants would agglutinate directly in heterologous sera.

On the basis of these findings it is recommended that Proteus X strains used in the Weil-Felix reaction be checked at least once a month to insure working with a nonmotile culture and at least once each quarter to exclude spontaneously occurring variants.

DIPHTHERIA: This Significance of the Schick Test in the Adult, Young, C. C., Bunney, W. E., Crooks, M., Cummings, G. D., and Forsbeck, F. C. Am. J. Pub. Health 24: 835, 1934.

A study of the relationship between the Schick reaction, antitoxin concentration, antigenic stimulation, and age in male adults, was made in two Michigan hospitals for the insane. The following observations were made from the data:

1. The results indicate that there is no antitoxin level in adults below which all Schick reactions are positive and above which all are negative.

2. Three-hundredths unit seems a satisfactory level to use as a dividing point. At any rate we are not able to suggest a better level.

3. "False negatives" and "false positives," basing these terms on the assumption that 0.03 unit or more means immunity and less than 0.03 unit susceptibility, occur in different proportions, before and after antigenic stimulation.